MOTOR NEURONE DISEASE ASSOCIATION

Abstracts from the

16th INTERNATIONAL SYMPOSIUM ON ALS/MND

Dublin, Republic of Ireland, 8-10 December 2005

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Introduction

Since the first international Symposium, on ALS/MND in 1990, we have witnessed considerable advances in our understanding of the disease. This meeting, organised by the Motor Neurone Disease Association in co-operation with the International Alliance of ALS/MND Associations, has become the premier forum for the presentation of new research into the condition.

When the International Symposium was last held in Dublin, ten years ago, the recently developed SOD1 transgenic mouse was opening up new avenues of exploration into degenerative mechanisms in ALS/MND and the imminent licensing of riluzole had revealed the first chink in the armour of the disease. To the outsider, the intervening period may not have seemed so productive - riluzole remains the sole licensed product - but for those who have devoted their careers to fighting this disease, the past decade has seen unprecedented advances in understanding the disease process, that will undoubtedly lead to significant treatment advances in the not-too-distant future.

The goal of the Programme Committee has been to attract plenary speakers of the highest international calibre who will give engaging and thought-provoking presentations on a range of topical themes. The Committee, chaired for the first year by Professor Pam Shaw, has aimed to shape a programme which includes cutting edge international research, highlighting new insights into disease pathogenesis and new approaches for improved treatments and management strategies for ALS/MND.

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Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 7–8

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SESSION 1 JOINT OPENING SESSION

C1 ALTERNATIVE MEDICINE- HYPE OR HOPE?

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'Alternative medicine' has become the politically correct term for questionable practices formerly labeled quack and fraudulent. During the past 15 years, many reports, even in medical journals, have contained no critical evaluation and have featured the views of proponents and their satisfied clients.

To avoid confusion, 'alternative' methods should be classified as genuine, experimental, or questionable. Genuine alternatives are comparable methods that have met science-based criteria for safety and effectiveness. Experimental alternatives are unproven but have a plausible rationale and are undergoing responsible investigation. The most noteworthy is use of a 10%-fat diet for treating coronary heart disease. Questionable alternatives are groundless and lack a scientifically plausible rationale. The archetype is homeopathy, which claims that 'remedies' so dilute that they contain no active ingredient can exert powerful therapeutic effects. Blurring these distinctions enables promoters of quackery to argue that because some practices labeled 'alternative' have merit, the rest deserve equal consideration and respect. Enough is known, however, to conclude that most questionable 'alternatives' are worthless.

The 'alternative movement' is part of a general societal trend toward rejection of science as a method of determining truths. In line with this philosophy, 'alternative' proponents assert that scientific medicine (which they mislabel as allopathic, conventional, or traditional medicine) is but one of a vast array of health care options. Instead of subjecting their work to scientific standards, they would like to change the rules by which they are judged and regulated. 'Alternative' promoters often gain public sympathy by portraying themselves as a beleaguered minority fighting a self-serving, monolithic 'Establishment'.

Under the rules of science, people who make the claims bear the burden of proof. It is their responsibility to conduct suitable studies and report them in sufficient detail to permit evaluation and confirmation by others. Instead of subjecting their work to scientific standards, promoters of questionable 'alternatives' would like to change the rules by which they are judged. 'Alternative' promoters may pay lip service to these standards. However, they regard personal experience, subjective judgement, and emotional satisfaction as preferable to objectivity and hard evidence. Instead of conducting scientific studies, they use anecdotes and testimonials to promote their practices and political maneuvering to keep regulatory agencies at bay.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045087

C2 PHENOTYPIC VARIATION IN MND/ALS

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'The fundamental law should be engrained that the starting point of all treatment is in a knowledge of the natural history of a disease'. William Osler, 1907.

Perhaps identifying a 'cure' for MND should be no more challenging than finding effective treatments for asthma, heart disease, cancer, epilepsy, or multiple sclerosis. All are heterogeneous disorders and in each case management relies largely on therapeutic agents that act on generic disease mechanisms and take little account of the complexities of individual variation. Our hope for MND is that common molecular mechanisms and therefore common therapeutic targets will link all forms of MND. Our fear is that new therapies will be effective only in sub-groups of people with rare sub-types of MND. The relevance of phenotypic variation in relation to the care of people with MND/ALS is evident in relation to symptom control, but it is not yet clear what heterogeneity means for disease modifying therapies – for example, riluzole. We do not know if there are responders and non-responders. The challenge is to find methods for understanding the molecular basis of clinical heterogeneity and to integrate such methods into clinical trials and epidemiology.

First we must understand the nature of clinical and genetic heterogeneity in MND. A disease entity is well exemplified by those forms of familial MND for which gene mutations have been identified such as familial MND associated with SOD1 or VAPB mutations, or linked to loci on chromosomes 9, 16, 18, or 20. Other MND genes remain to be localized to a chromosomal locus. All these examples could be said to be different diseases, although all share some or all of the typical clinical features of MND. While some SOD1 gene mutations are associated with a stereotyped phenotype, others are not. Genetic background appears to influence phenotype (e.g. in D90A homozygous familial MND). Specific gene therapy for people with SOD1 gene mutations is being developed. The same approach may be needed for the other gene mutations, at huge effort and cost and for the benefit of relatively few individuals at risk.

There is also striking phenotypic variation in sporadic MND/ALS. The differences in prognosis between bulbar onset and limb onset disease are well known, but it is important to recognize other MND syndromes, such as the 'flail arm' and 'flail leg' syndromes, and lower motor neuron forms of MND (PMA- progressive muscular atrophy). The presence of cognitive change in a significant proportion of people with MND is now apparent. This was hardly discussed back in 1990. These phenotypic variations must be recognized and their biological basis better understood if physicians are to tailor advice on disease

progression, prognosis, drug therapy, and care to the needs of the individual.

How can this be achieved? Undoubtedly we are missing opportunities to understand the biological basis of these important variations within the rubric of MND/ALS by our failure to pool understanding and resources nationally and internationally. Large-scale clinical trials and prospective epidemiological and natural history studies can furnish prospectively acquired clinical information linked with DNA and pathology resources that will be necessary to attack these issues. Hundreds of MND patients and controls – most likely thousands – are needed if this approach is to succeed.

We should now rapidly advance beyond the era of anecdote and small studies to building international collaborations that will allow us, in time, to understand the molecular basis of MND in its various forms – familial, sporadic, bulbar onset and limb onset, flail arm and flail leg, with and without cognitive impairment and so on. At the same time we should develop new methods to understand individual and sub-group responses to disease-modifying treatments. The arrival of a generic therapy that halts disease progression in all forms of MND may of course obviate all this effort. Let us hope so. But perhaps, like AE Houseman, we should: 'Do as a wise man would, and train for ill, and not for good'.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 9–12

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SESSION 2A MOTOR NEURON BIOLOGY AND PATHOLOGY

C3 PATHOLOGICAL VARIATION IN MOTOR NEURON DISEASE

INCE P

No abstract available

C4 IS THERE A CAUSAL ROLE OF STERYL GLUCOSIDE NEUROTOXINS IN ALS?

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Background: The causal factor(s) responsible for sporadic neurological diseases are unknown and the stages of disease progression remain undefined and poorly understood. Epidemiological studies of the Guamanian variant of ALS, amyotrophic lateral sclerosis-Parkinsonism dementia complex (ALS-PDC) have shown a positive correlation between consumption of washed cycad seed flour and the development of the disease. *In vivo* studies in which adult male mice consume washed cycad seed flour as part of diet show that treated animals show profound and progressive motor, cognitive, and olfactory behavioural deficits combined with the loss of neurons in each of the respective neural subsets. The expression of these outcomes mirrors precisely the behavioural and pathological deficits in ALS-PDC. *In vitro* experiments using isolated cycad fractions have identified the likely neurotoxins as variant steryl glucoside molecules contained in washed cycad flour, specifically β-sitosterol β-D-glucoside (BSSG), campesterol β-D-glucoside, and stigmasterol β-D-glucoside.

Objectives: To determine if steryl glucoside molecules are causal agents in ALS-PDC and if similar molecules can be identified in plasma from ALS patients in North America.

Methods: BSSG was fed to mice as part of their diet for 10 weeks. Mice were tested using an established battery of behavioural tests and histology previously used to observe neurological deficits and pathology. Screening for these putative molecules was conducted with human ALS and control patients. HPTLC was used to measure steryl glucoside concentration in 40 ALS and 30 control plasma samples.

Results: *In vivo* experiments with mice fed BSSG show a significant 25% loss of motor neurons in the lumbar spinal cord with increased caspase-3 labelling. In human samples, initial results show increased steryl glucoside concentrations in ALS patients compared to controls.

Discussion and conclusions: The identification of steryl glucosides as neurotoxins in the animal model and the preliminary data suggesting elevated levels of these molecules in human neurological disease warrant further investigation of steryl glucoside as causal neurotoxins or as biomarkers of disease status or progression.

C5 MUTANT HEAT SHOCK PROTEIN 27 RESULTS IN THE SELECTIVE AGGREGATION OF SPECIFIC CELLULAR COMPONENTS

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Background: Recently there has been a dramatic increase in the identification of genes in which mutations lead to isolated motor neuron degeneration. The hereditary motor neuropathies (distal spinal muscular atrophies) are a heterogeneous group of pure lower motor neuron degenerative disorders for which several causative genes including heat shock protein 27 (Hsp27) have been identified. Hsp27 is a molecular chaperone which has a number of different roles within cells and has been shown to be essential for the survival of motor neurons.

Objectives: To develop a cell culture model to examine the molecular mechanism whereby mutant Hsp27 leads to motor neuron degeneration.

Methods: We have developed an assay using transfected primary cultures to investigate the effects of wild-type (wt) and mutant (P182L) Hsp27 on the distribution and transport of a number of cellular components.

Results: We have demonstrated that mutant but not wt Hsp27 abnormally aggregates in the cell bodies of primary cultures. These aggregates of mutant Hsp27 then result in the sequestering of wt Hsp27 within cell bodies. In addition, selective components of the neuronal cytoskeleton such as neurofilaments, but not tubulin, are also found to co-aggregate with mutant Hsp27. Furthermore, a component of the dynein/dynactin complex which is essential for retrograde transport was also found to aggregate with mutant Hsp27 in cell bodies. The distribution of mitochondria which are known to be transported within axons was not affected by mutant Hsp27, suggesting selective disruption of the transport of certain cellular components.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045096

Discussion: Using a primary cell culture based assay we have demonstrated that mutant but not wt Hsp27 appears to result in the selective aggregation of specific cellular components. To extend these *in vitro* studies, we have generated transgenic mice overexpressing mutant Hsp27. We are currently in the process of trying to identify further targets, the transport of which may be disrupted by mutant Hsp27. In addition we are further investigating the role of wt Hsp27 in axons. The identification of the specific targets disrupted by mutant Hsp27 may increase our understanding of the mechanisms whereby motor neurons are selectively vulnerable.

C6 HEAT SHOCK RESPONSE, BUT NOT Hsp27, PREVENTS MUTANT SOD1-DEPENDENT CELL DEATH IN A CULTURE MODEL OF ALS

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Background: Amyotrophic lateral sclerosis (ALS) is characterized by selective loss of motor neurons in the spinal cord, brainstem and motor cortex. Mutations in the free-radical scavenging copper-zinc superoxide dismutase (SOD1) enzyme underlie one form of familial ALS. The pathogenic mechanism of these mutations is elusive but is thought to involve oxidative stress and abnormal protein aggregation. These two phenomena are known to induce heat shock proteins (Hsps) which protect stressed cells through their chaperoning and anti-apoptotic activity.

Objectives: We previously showed that Hsp27 is up-regulated in the spinal cord of mutant SOD1 mice. This study was conducted to evaluate the possible protective role of Hsp27, using an *in vitro* model of mutant SOD1 and WT SOD1 overexpressing neuro2a (N2a) mouse neuroblastoma cells.

Methods: N2a cells were exposed to cyclosporine A, lactacystin or a severe heat shock and assessed for cell survival using MTS assay. Transfection with an Hsp27 construct was performed using Amaxa transfection. For endogenous up-regulation of Hsp27, these cells were exposed to a pre-conditioning heat shock. Blocking of this endogenous up-regulation of Hsp27 was achieved with RNAi technology. Western blotting was carried out to assess the expression of SOD1, as well the levels of Hsp27 before and after transfection with siRNA. Immunohistochemistry was also performed to study the efficacy of the siRNA treatment.

Results: Mutant SOD1 dependent differential cell death could be induced by heat shocking the cells, but also by treating the cells with cyclosporine A and lactacystin. Transfection of the cells with an Hsp27 construct did not protect mutant SOD1 cells from differential death. However, pre-conditioning N2a cells with a mild heat shock was accompanied by a significant up-regulation of Hsp27 in the mutant SOD1 cells, and protected these cells against subsequent mutant SOD1-dependent cell death induced by a more severe heat shock. Selective inhibition of this Hsp27 up-regulation, through the use of Hsp27 siRNA, did not attenuate the protective effect of this treatment.

Conclusions: These results suggest that activation of the heat shock response can provide the necessary defences for cells with mutant SOD1 to withstand other stresses, and that this protection is probably mediated through a complex cohort of molecules of the stress response pathway of which Hsp27 is not an essential component.

C7 ONSET ADMINISTRATION OF MOTEXAFIN GADOLINIUM EXTENDS SURVIVAL IN G93A MICE: POSSIBLE ROLE OF INCREASED ZINC OCCUPANCY

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Background: ALS-associated SOD1 mutants, including G93A SOD1, have been shown to have lower Zn binding affinity; Zn-deficient SOD1 has both pro-oxidant and pro-aggregatory properties, but the presence of Zn-deficient SOD1 *in vivo* has yet to be demonstrated. Motexafin gadolinium (MGd) is a novel drug that has been shown to increase intracellular concentrations of free Zn in cancer cell lines.

Objectives: To measure the *in vivo* metallation state of G93A SOD1 from spinal cord and to assess the magnitude and mechanism(s) of the neuroprotective effect of MGd.

Methods: Rapidly purify G93A SOD1 mutant from transgenic spinal cord under mild, non-denaturing conditions in the presence and absence of low abundance Zn isotope (Zn67) and measure Zn67 enrichment via ICP-MS. Administer MGd at symptom onset to G93A transgenic mice and examine survival and proteomic changes after 10 days (relative to age-matched vehicle-treated controls), as well as assess ultimate survival.

Results: G93A SOD1 mutant purified from spinal cord at four time points, from symptom onset to end stage, revealed a progressive increase in Zn67 enrichment, indicating that Zn binding sites in the mutant enzyme were unoccupied at the time of tissue disruption. Onset administration of MGd (1 mg/kg/day IP) to G93A mice resulted in up to a 2.5-fold increase in survival after onset, relative to vehicle-treated littermate controls. Preliminary proteomic analysis of cohort mice treated for 10 days revealed decreased expression of the Zn binding protein NF-L, increased expression of NF-H, and alterations in important mitochondrial enzymes.

Discussion and conclusions: Using a novel method that we developed for measuring Zn binding site occupancy of Zn-binding proteins, we offer the first compelling evidence for the presence of Zn-deficient SOD1 mutant in the spinal cord of transgenic animals. Furthermore, the levels of Zn-deficient SOD1 mutant increase over time and correlate with disease severity. Using a clinically relevant onset administration paradigm, MGd produces a marked and reproducible increase in survival of G93A mice. Changes associated with the therapeutic effect suggest that could be related, in part, to changes in Zn uptake and handling by neurons, i.e.increases in intracellular free zinc, combined with decreases in the major Zn binding protein in neurons (NF-L), could result in more available Zn and a net decrease in the amount of toxic Zn-deficient SOD1 present. In any case, lowering of NF-L expression and increased NF-H expression, in and of themselves, have been shown to produce an increase in survival in G93A mice. Overall, these results suggest that MGd could be a useful therapeutic agent in all forms of ALS.

C8 GLUR2 DEFICIENCY ACCELERATES MOTOR NEURON DEGENERATION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is characterized by a progressive degeneration of motor neurons. Familial ALS accounts for 10% of all cases and in about 20% of the familial cases, mutations of the superoxide dismutase 1 (SOD1) gene have been identified. Transgenic mice overexpressing human mutant $SOD1^{G93A}$ develop a progressive motor neuron disorder and provide a good model for familial ALS.

AMPA receptor-mediated excitotoxicity has been implicated in the selective degeneration of motor neurons in ALS. Excessive stimulation of AMPA receptors is particularly toxic to motor neurons *in vitro* due to Ca^{2+} influx through AMPA receptors. One of the factors underlying this selective vulnerability of motor neurons is the presence of a large proportion of Ca^{2+}-permeable (i.e. GluR2-lacking) AMPA receptors. However, the precise role of GluR2-lacking AMPA receptors in motor neuron degeneration remains to be defined. We therefore studied the impact of GluR2-deficiency on motor neuron death. Cultured motor neurons from GluR2 deficient embryos displayed an increased AMPA receptor Ca^{2+} permeability, an increased Ca^{2+} influx upon AMPA receptor stimulation and an increased vulnerability to AMPA receptor-mediated excitotoxicity. To investigate the role of GluR2 in motor neuron degeneration *in vivo*, we crossbred mutant $SOD1^{G93A}$ mice with GluR2 knock-out mice. GluR2 deficiency clearly accelerated the motor neuron degeneration and shortened the lifespan of mutant $SOD1^{G93A}$ mice. These findings indicate that GluR2 plays a pivotal role in the vulnerability of motor neurons *in vitro* and *in vivo* and that therapies that limit Ca^{2+} entry through AMPA receptors might be beneficial in ALS.

C9 ALTERED EXCITABILITY OF CORTICAL NEURONS IN A TRANSGENIC MODEL OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background: Abnormal balance between intracortical inhibitory and excitatory mechanisms has been found to contribute to the genesis of motor cortex hyperexcitability in amyotrophic lateral sclerosis (ALS). Transcranial magnetic stimulation (TMS) has shown that both increased excitability of cortical motor neurons and reduced intracortical inhibition contribute to observed motor cortex hyperexcitability in ALS patients.

Objectives: The purpose of the present study is to investigate if cortical neurons excitability is altered in a transgenic model of ALS and if this hyperexcitability is due to intrinsic properties of the single cortical neuron.

Methods: We have grown cortical neurons in cultures from transgenic mice expressing G93A mutant SOD1. We employed non-transgenic mice (control), transgenic mice expressing high levels of the human wild-type protein (SOD1), and transgenic mice expressing high levels of the human mutated protein ($Gly^{93}\rightarrow$Ala, G93A) to study and compare cortical excitability and passive membrane properties. Embryos at day 15 of gestation were used. All transgenic progenies were screened for the presence of the human SOD1 enzyme. Cells were used between 10 and 12 days *in vitro* and for the electrophysiological recordings we used the whole-cell configuration of the patch-clamp technique.

Results: The passive membrane properties, the pattern of repetitive firing and the action potential properties were examined in cortical neurons. In all tested cells, the injection of -0.1 nA current (200 ms) from the membrane potential of -60 mV evoked a hyperpolarizing response that reached a steady-state used to calculate the membrane time constant and input resistance of each cell. The resting membrane potential, the input resistance, the membrane time constant and the cell capacitance of control, SOD1 and G93A cortical neurons did not present significantly different values. The injection of +200 pA failed to elicit multiple actions potential in all the neurons. In all tested cells, the time interval between the first two spikes of a train was decreased when the amplitude of the train evoking current was increased. For the injected currents equal to +200 pA, the firing frequency in the G93A culture was significantly greater (37 ± 10 Hz, $n=14$; $p<0.05$) than that in the control (25.7 ± 8.3 Hz, $n=17$) and in the SOD1 (29 ± 7.6 Hz, $n=16$). The threshold for

the action potential onset was decreased significantly ($p<0.05$) in the G93A neurons (-36.6 ± 4.7 mV) compared to control (-31.7 ± 3.4 mV) and SOD1 (-32.4 ± 4.3 mV).

Discussion and conclusions: These data show that the membrane passive properties of cortical neurons are unaffected by the overexpression of the human transgene, either wild-type or G93A. On the contrary, the firing frequency and the action potential onset were altered in G93A cortical neurons, indicating an intrinsic neuronal hyperexcitability that may contribute to the pathogenesis of the disease.

Acknowledgements: This work is supported by a grant from MIUR (Firb: RBAU01A7T4), Italy.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 13–16

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SESSION 2B CLINICAL TRIALS AND TRIAL DESIGN

C10 PHASE II/III CONTROLLED TRIAL OF TCH 346 IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: TCH 346 exerts anti-apoptotic effects by binding to the glycolytic enzyme, glyercerol-aldehyde-3-phosphate dehydrogenase (GAPDH) and blocking the apoptotic pathway in which GAPDH is involved. Apoptosis is theorized to be a key pathogenic event in neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). Therefore, a dose-finding Phase II/III study of the safety and efficacy of TCH 346 in ALS was undertaken.

Objectives: To determine whether TCH 346 at four double-blind fashion to receive either placebo or one of four oral doses of TCH 346 (1.0, 2.5, 7.5 and 15 mg/d) administered once daily for up to 44 weeks. The primary outcome measure was the rate of change in the ALS Functional Rating Scale - Revised (ALSFRS-R) under treatment. The novel trial design included a 16-week natural history lead-in phase to determine each patient's rate of disease progression, and the between-treatment comparison was adjusted for the individual pre-treatment rates of progression. The study was powered to detect a 25% reduction in the rate of functional decline of the ALSFRS-R compared to placebo. Secondary outcome measures included adverse events, survival, defined as time to death, tracheostomy, intubation, or ventilation, as well as functional outcomes measures: pulmonary function and manual muscle strength (MMT).

Results: Five hundred and ninety-one patients were enrolled at 42 sites in Europe and North America. The last patient completed the study on 14 December 2004. There were no significant differences in baseline variables. There were no differences between placebo- and active-treatment groups in the mean rate of decline of the ALSFRS-R or in the secondary outcome measures (serious adverse events, survival, pulmonary function and MMT).

Conclusion: These data provide no evidence of a beneficial effect of TCH 346 on disease progression in patients with ALS. These data might provide useful information on novel study designs for future clinical trials in ALS.

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045104

C11 MULTI-CENTER CLINICAL TRIAL OF CREATINE MONOHYDRATE IN PATIENTS WITH ALS

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Background: Creatine monohydrate has been shown to be neuroprotective in mouse models of motor neuron disease. A small (n=21) pilot study of creatine monohydrate in ALS showed a significantly greater improvement in strength and/or a more modest decline in strength compared with patients receiving placebo. By contrast there have been two negative placebo-controlled studies in the ALS patient population. The subjective reported benefit of creatine remains and may be due to either improved strength or decreased muscle fatigue. Muscle fatigue was not accessed in any of the prior trials. We will report the final results of our multi-center trial (NIH supported) using creatine in patients with ALS. Multiple outcome measures, including muscle fatigue will be reported.

Objectives: To determine whether treatment with creatine monohydrate results in an acute increase in muscle strength (within three weeks) and whether that effect is sustained with chronic therapy. Also, to determine whether the effects of muscle fatiguability can be decreased with the acute and chronic use of creatine monohydrate.

Methods: One hundred and seven patients were enrolled at nine sites and randomized at a 1:1 ratio to receive either creatine monohydrate or placebo. Patients received a loading period of 5 mg study medication twice daily for five days followed by 5 mg daily for nine months. Patients included in the study were diagnosed with laboratory-supported, probable, or definite ALS, had a disease duration of less than five years and at least 5 of 10 upper extremity muscle groups with a MRC grade of 4 or better. The slope of change of upper extremity strength using maximal voluntary isometric contraction (MVIC) as well as fatigue index measured by sustained MVIC will be analysed. Secondary endpoints include forced vital capacity (FVC), ALS-FRS, quality of life (SF-12), and safety and tolerability.

Results: Patient follow-up is expected to be completed in October 2005. Currently, creatine monohydrate has proven to be safe and well tolerated by evaluation of the data safety and monitoring committee. We anticipate positive effects on muscle fatigue which may account for the frequent reported subjective benefits. In addition, distinctions between this study and the prior controlled trials will be discussed. If the significant acute benefits of treatment (seen in the pilot study) are not sustained, implications of alternative dose cycling will be discussed.

Conclusion: Based on preclinical and pilot data, creatine monohydrate appears to be an important therapy for consideration in the treatment of ALS patients. Complete data including the novel approach of measuring muscle fatigue in a clinical trial as well as the five years of experience with the study of creatine monohydrate will be presented.

C12 RANDOMIZED CONTROLLED PHASE II TRIAL OF GLATIRAMER ACETATE (COPAXONE) IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: One potential mechanism of neurodegeneration in ALS involves a brisk neuroinflammatory reaction. Pro-inflammatory factors, seen in human and mouse models of ALS, affect primary and secondary neurodegenerative processes. We posit that vaccination to brain self-antigens can activate anti-inflammatory Th-2 cells, which migrate to points of inflammation where they serve as a source of inhibitory cytokines and neurotrophic factors. Vaccination with glatiramer acetate (GA) boosts anti-self T-cell-mediated immunity, is FDA approved for treatment of multiple sclerosis, and delays disease progression in animal models of neurodegenerative disorders, including ALS.

Objectives: To examine the safety, tolerability and immunologic effects of GA in ALS.

Methods: We conducted a six-month randomized controlled trial in 30 patients meeting El Escorial criteria for ALS. Patients were assigned to GA 20 mg sc daily, GA 20 mg sc bi-weekly or an untreated control group, and received monthly evaluations. Record of adverse events (AE); vital signs; liver and renal function; blood counts; electrocardiogram; the ALS Functional Rating Scale-Revised (ALSFRS-R); manual muscle testing; forced vital capacity; and Timed Get Up and Go Test were used as measures of safety. Tolerability was assessed by the ability to complete the trial on the assigned treatment. T-cell proliferation assays were used to assess immune response. Evaluators were blinded to treatment assignment. The trial, which received FDA IND and IRB approval, was 80% powered (alpha=0.05) to detect a 16% difference in levels of inhibitory Th-2 cells. Differences were determined using χ^2 tests, ANOVA and Mixed-models ANOVA.

Results: There were no differences in baseline characteristics between groups, including site of disease onset, riluzole use and ALSFRS-R scores. At study conclusion, five patients in each active treatment group had at least one injection site reaction ($p=0.01$). A systemic post-injection reaction consisting of palpitations, flushing, and thoracic tightness occurred in five of the treated patients ($p=0.15$). There were no other differences in safety measures between groups, or in treated patients overall compared to controls. There were six serious adverse events (two in the daily, one in the bi-weekly and three in the control groups, respectively), including four deaths; none was considered related to GA. There were no dropouts or dose reductions for reasons other than death. Analysis of lymphoproliferative responses to GA in *in vitro* assays showed significant effects by treatment group ($p=0.006$), visit ($p=0.018$) and in interaction between dose and visit ($p=0.012$).

Conclusions: We report the first randomized controlled trials of vaccination therapy in ALS. GA was safe and well tolerated in two different doses, and with riluzole. There were no significant differences in any safety measures other than injection reactions. The robust T-cell mediated immune response provides evidence of enhanced protective immunity, and will be used to select dosing frequency in a trial of efficacy.

C13 NOVEL PHASE II DESIGN FOR CLINICAL TRIALS OF ALS USING SELECTION PARADIGM AND GROUP SEQUENTIAL ANALYSIS

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Background: Many drugs have promising neuroprotective properties and could be tested in ALS. Once the scientific rationale for an agent is established, clinical trials are currently conducted sequentially on single medications, and each may take a decade or longer. Moreover, the low prevalence of ALS limits the number of agents that can be tested by this method. A novel more efficient means of clinical drug screening is needed. The selection trial design allows the simultaneous study of multiple medications with reduced sample size, by identifying a superior treatment if it exists with a pre-specified high Probability of Correct Selection (PCS). Data are sampled until a weight of evidence is acquired or a maximum sample size is recruited. The goal is to select the best from a group of agents for testing in phase III trials.

Objective: To present a phase II trial design that performs efficient screens of multiple drugs simultaneously.

Methods: We chose change in ALSFRS-R over six months as the primary endpoint. Using pilot data from 163 patients, we determined that the six-month decrease in ALSFRS-R has a mean of 5.82 with SD of 6.77. Computer simulation was used to create a two-armed, group-sequential trial that meets the following criteria: 1) at least 80% PCS when one agent truly reduces the mean decline in ALSFRS-R by 20% compared to the other; 2) feasible sample size; 3) realistic trial duration.

Results: In this design, patients are randomized in pools of 60 to one of two arms. Not more than two pools (120 patients) are needed. After the first pool completes six months, the trial is stopped if the mean difference between the two arms is adequately large (defined as 0.75 times the SD); the agent inducing the smaller mean change in ALSFRS-R is selected. Otherwise, an additional 60 patients are randomized. If an agent provides 20% improvement in the mean decrease, the trial will conclude after one pool with 67% probability. We estimate a four-month recruitment period for 60 patients, requiring 10 months to complete each pool. Should the trial need two pools, it will take approximately 20 months. Even if no agent truly reduces the mean decline in ALSFRS-R by 20%, the selection procedure will still select the better treatment with higher probability than others. Three two-armed trials can be conducted concurrently with 360 patients. It is possible, therefore, to obtain data on six agents, selecting three best performing drugs in a 20-month period. If sites outside the US are included, the trial could be expanded to provide data on 12 drugs (720 patients).

Conclusions: Compounds with sufficient scientific rationale can be compared head-to-head in concurrent randomized selection trials designed to determine with high probability the best agent from among a group of therapies for ALS. Group sequential trial design further enhances efficiency by allowing early termination.

C14 NEW PHASE II DESIGNS TO IMPROVE CLINICAL TRIALS IN ALS

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Background: Phase II trials, where small numbers of patients all receive treatment and are compared to historical controls, are of interest because of their efficiency. A non-inferiority trial (sometimes called a futility trial) is powered to screen out treatments that are inferior to current practice. This is in contrast to the efficacy trial where the goal is to establish efficacy. The non-inferiority trial gains its efficiency by proposing a level of superiority for the treatment under the null hypothesis. If the trial results fail to reject this hypothesis (i.e. the treatment is not inferior to current practice), a phase III is recommended. Efficiency might be enhanced by conducting the trial sequentially.

Objective: To determine sample sizes required by different phase II designs and to compare their efficiencies.

Methods: We used ALS-FRS data from a randomized trial of gabapentin to estimate rates of decline and sources of variation in patients with ALS. These estimates were used to determine sample sizes for non-inferiority type phase II trials. We considered fixed sample size and sequential trial designs. The two-stage sequential design allows stopping the trial early by making one interim comparison. A 'Christmas Tree' sequential design allows comparisons after each patient has completed the trial.

Results: We found that the average rate of decline (slope) for ALSFRS patients was 0.84 units per month (ALS-FRS scale 0–40, with 40 indicating no deficits). The standard deviation for this rate of decline based on the first six months of data for each patient was estimated to be 0.67 for the between patient component and 0.06 for the within patient component (based on six months follow-up). Results based on these estimates are summarized below:

Table. Sample sizes required for single arm trials based on ALSFR-S.

	Efficacy Trial	Non-inferiority Trial		
	H0: No effect	H0: Reduction		
	H1: Reduction	H1: Increase		
	α=0.05 p=0.90	α=0.10 p=0.95		
Slope Reduction/ Increase	Fixed sample size	Fixed sample size	2 Look sequential design[1]	Xmas tree sequential design[2]
15%	272	75	58	46
20%	153	42	32	26
25%	98	27	21	17

[1]Expected sample size under alternate hypothesis: Table 4.3 in Jennison and Turnbull.
[2]Expected sample size under alternate hypothesis: Table 4.7 in Jennison and Turnbull.

Conclusions: Inferiority/superiority trials result in substantial savings in sample size. Additional savings in sample size may occur when trials are conducted sequentially.

C15 CLINICAL TRIALS IN ALS CAN BE SHORTENED BY SELECTING RAPIDLY PROGRESSING PATIENTS

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Background: We have previously shown that ALS-FRS, motor unit number estimation (MUNE) and the neurophysiological index (NI) are the most sensitive measurements in evaluating progression of ALS patients. When following a group of patients a significant change is recorded at three months for each of those measurements, but not at one month.

Objectives: We decided to test if ALS-FRS could be used to select a population of patients with rapid disease progression when applied during a three-month lead-in period.

Methods: From a population of 57 ALS patients evaluated in a three-month lead-in period with the ALS-FRS scale, we selected a subgroup of patients fulfilling the following criteria: ALS-FRS >30 at entry; ALS-FRS decline over this period $>8.36\%$, representing those outside the 80th percentile; disease duration <24 months; forced vital capacity $>60\%$ of the predicted value at entry; regular medication with riluzole. A subset of 12 patients (seven were females) fulfilled these criteria (mean age 59 years, range 47–69 years); mean disease duration 8.3 months (range 2–20 months), eight had probable and four had definite ALS. In this group, the mean ALS-FRS score was 34.7 (SD 1.7, range 32–37) at baseline and 29.7 (SD 2.9, range 23–33) at the end of this lead-in period. One month and three months later each patient was assessed, using ALS-FRS, MUNE and NI. Comparison was made with a group of 21 patients with slower progression, from the remainder of the original cohort.

Results: ALS-FRS, MUNE and NI changed significantly at one month and three months ($p<0.01$) in this group of 12 patients with rapidly progressing disease. In the remaining patients no change was observed at one month, but at three months ALS-FRS, MUNE and NI showed a significant change.

Conclusions: ALS-FRS can be applied in a three-month lead-in period to select ALS patients with very rapid progression. This method of selecting a population of ALS patients could be useful in designing short-term and low-cost clinical trials to test new drugs.

C16 PREDICTING RATE OF DISEASE PROGRESSION USING PERCENT OF PREDICTED NORMAL STRENGTH IN ALS CLINICAL TRIAL DESIGN

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Background: During the past decade there has been a huge increase in the number of potential therapeutic agents for the treatment of ALS. However, the ability of the clinical trials community to test these agents remains limited due to cost, time, and especially resource constraints. More sensitive and accurate outcome measures could improve clinical trial efficiency by allowing fewer subjects to be tested in a shorter amount of time.

Another challenge to clinical trial efficiency is the need for placebo groups. Although the course of ALS is remarkably linear within each patient, disease progression can vary up to 10-fold between patients. Thus, if a predictable rate of disease progression could be established for each subject, subjects could be used as their own, highly matched, controls. This could potentially eliminate the need for a placebo crossover arm in clinical trials.

Objective: To determine how many monthly data points are needed to accurately predict the rate of disease progression over a one-year period.

Methods: Using an ALS natural history databank containing almost 5000 visits, we examined data of subjects who had at least one year of strength data using maximal voluntary isometric contraction (MVIC). MVIC data were then converted to percent of predicted normal (PPN) using regression equations based on age, gender, height and weight. For each subject, the PPN slope was determined using data collected on a monthly basis over a full year. Then, we compared the slope using one year of data to the slope based on the first two monthly data points, then three monthly data points, etc. The correlation, residual, and mean square error between the actual slope, based on a year's data and each slope yielded from 2, 3, 4, 5, etc. months of data were calculated.

Results: Results showed data collected over a five-month period were very highly correlated with the full year's data ($r=0.82$), yielding a residual of 0.58 and a mean square error of 16.3. Less than five data points was less predictive of the full year's slope. More than five months of data yielded only slightly better ability to predict the slope over a full year.

Discussion and conclusions: Our data suggest that a very predictable slope is yielded from MVIC converted to PPN with as few as five monthly data points. This finding raises the possibility that we may be able to design ALS clinical trials using subjects as their own controls. More analysis is needed to confirm whether this method may improve clinical trial efficiency by eliminating the need for placebo controls and thereby allowing fewer subjects be tested over a shorter time period.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 17–18

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SESSION 3A LESSONS FROM OTHER MOTOR NEURON DISORDERS

C17 DISTAL HEREDITARY MOTOR NEUROPATHIES: CLINICAL AND MOLECULAR GENETIC ASPECTS

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The distal hereditary motor neuropathies (distal HMN) are a clinically and genetically heterogeneous group of disorders. The predominant or exclusive involvement of motor axons and motor neurons links them to other motor neuron disorders such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). There are however important clinical differences that may point to distinct underlying pathomechanisms. In distal HMN, weakness and atrophy start in the distal parts of the lower limbs suggesting a length-dependent mechanism. The involvement is symmetrical and bulbar muscles remain clinically intact. The onset is usually in the first or second decade of life; evolution is slowly progressive and life expectancy is not compromised. In some distal HMN subtypes involvement of upper motor neurons and corticospinal tracts is evidenced by the presence of pyramidal tract signs. Intriguingly, mutations in particular genes may result in either distal HMN or hereditary motor and sensory neuropathy (HMSN) and sometimes both phenotypes run in different branches of a family, suggesting a role for modifiers. Molecular genetic studies have identified several genes involved in distal HMN. These genes include: immunoglobulin μ-binding protein2 (IGHMBP2) in distal HMN type VI or spinal muscular atrophy with respiratory distress type I (SMARD1); the small heat shock proteins HSP22 and HSP27 in juvenile onset distal HMN type II; glycyl tRNA-synthetase (GARS) in distal HMN with predominant involvement of upper limbs (distal HMN type V); the Berardinelli-Seip congenital lipodystrophy (*BSCL2*) gene in distal HMN with upper limb predominance and spasticity (Silver syndrome); dynactin (DCTN1) in distal HMN with vocal cord paralysis (distal HMN type VII), senataxin (SETX) in ALS 4 or distal HMN with pyramidal tract signs. The seven distal HMN related genes identified so far have diverse functions but some common themes emerge: IGHMBP2 as the *SMN1* gene (proximal SMA) is involved in the pre-mRNA splicing machinery and the *SETX* gene has a helicase activity and is also important for RNA processing; mutations in GARS could affect protein synthesis; axonal transport is the function of the DCTN1; the mutant small HSPs 22 and 27 may lead to dysfunction of the axonal transport and dysregulate the cytoskeleton causing motor neuron death, mutations in the small *HSP* genes, as well as in the *BSCL2* gene, also result in aggresome formation suggesting a role for efficient protein folding. These common themes may point to mechanisms that could be involved, either primarily or secondary, in motor neuron death in other motor neuron disorders such as ALS.

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045113

C18 SPINAL MUSCULAR ATROPHY

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Classical spinal muscular atrophy (SMA) is caused by mutations in the *SMN1* gene on human chromosome 5. The corresponding gene product that is also produced at low levels from a second SMN gene (*SMN2*), is part of a multiprotein complex involved in the assembly of spliceosomal snRNP complexes. The question of why reduced levels of the ubiquitously expressed SMN protein cause specific motor neuron degeneration without affecting other cell types is not fully solved. Two hypotheses have been put forward: 1) Loss of the classical function of the Smn complex is particularly detrimental to motor neurons, and 2) The Smn complex serves additional functions in motor neurons, i.e. in the context of axonal mRNA transport and presynaptic mRNA translation.

Gene knockout mice for Smn and Gemin2 have been established. Whereas complete knockout of Smn and Gemin2 is lethal during early development, expression of low levels of Smn protein from a transgenic SMN2 copy leads to classical motor neuron symptoms during post-natal development. Gemin2 heterozygous mutant mice do not develop motor neuron disease. The Smn protein interacts with many additional proteins, including RNA helicase A, snoRNPs, and various members of the hnRNP family, including hnRNP R and hnRNP Q. These proteins colocalize with Smn in motor axons.

Isolated motor neurons from Smn-/- SMN2 tg mice exhibit normal survival in the presence of neurotrophic factors but their axon growth is disturbed. In differentiating PC12 cells, overexpression of Smn or its binding partner hnRNP R enhances neurite growth, indicating that a complex of Smn and hnRNP R is involved in the regulation of axon growth and maintenance. Knockdown of Smn in zebrafish results in axonal abnormalities in motor axons, and similar observations have been made after knockdown of hnRNP R in zebrafish. Reduced axon growth in Smn deficient motor neurons correlates with reduced ß-actin mRNA and protein levels in distal axons and growth cones. HnRNP R specifically binds to the 3'UTR of ß-actin mRNA *in vitro*. This interaction depends on the presence of Smn protein. These findings indicate that SMA is not

caused by a primary cellular defect that interferes with cellular survival but with a defect in axon growth and maintenance. In this respect, SMA resembles other forms of motor neuron disease which are caused by defects in axonal transport (i.e. mutations in the dynactin gene).

The current status of research suggests that therapeutic intervention is possible on two levels: 1) up-regulation of SMN protein expression from the *SMN2* gene, and 2) up-regulation of axonal mRNA transport and local protein synthesis in the presynapse in order to compensate for the functional deficits caused by SMN deficiency in SMA. Both pathways are currently followed, both in mouse models and in early clinical studies.

C19 EXPERIMENTAL ENHANCEMENT OF AXONAL REGENERATION, COMPENSATORY NEURITE GROWTH AND FUNCTIONAL RECOVERY IN THE ADULT INJURED MAMMALIAN SPINAL CORD

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Although adult CNS circuits have a high degree of stability, a transitory activation of growth associated genes paralleled by regenerative and compensatory sprouting processes can be observed in the adult brain and spinal cord in response to different kinds of injuries. The length of growing axonal branches is often restricted to less than 1 mm. Neurite growth inhibitory factors are present in the adult CNS, particularly in white matter (e.g. Nogo-A, MAG, ephrins, semaphorins, proteoglycans). Neutralizing antibodies against Nogo-A or Nogo-A KO significantly decrease the inhibitory nature of CNS tissue for neurite outgrowth *in vitro*. *In vivo*, transected corticospinal axons in the adult rat spinal cord elongate over distances up to greater than 1cm in animals infused with neutralizing antibodies against Nogo-A or following blockade of Nogo-A receptor interaction. These animals show an impressive enhancement of functional recovery in motor performance and sensory functions, in the absence of changes in the pain threshold. Enhancement of sensory-motor recovery was also observed in adult macaque monkeys with high cervical hemisections: precision movements of hand and fingers were restored to a high degree in the animals treated for four weeks with intrathecal anti-Nogo-A antibodies. In addition to long distance regenerative axon growth, enhanced compensatory growth of intact fibres of different spinal tract systems was observed. These studies point to a much higher level of endogenous plasticity of the adult CNS including the spinal cord than commonly assumed. The neurite growth inhibitor Nogo-A seems to play a crucial role in restricting this plasticity to short spatial dimensions, a mechanism that may serve to stabilize the complex wiring of the intact normal adult CNS.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 19–20

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SESSION 3B QUALITY OF LIFE AND PALLIATIVE CARE

C20 NEW APPROACHES TO PALLIATIVE CARE

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Background: The development of palliative care owes much to the vision and commitment of some extraordinary people. Research on appropriate content and delivery of palliative care and evidence on its effectiveness have been limited. This is both because of reluctance to challenge some of the core ideas of palliative care, but also because it is genuinely difficult. This paper presents some findings from three related studies on palliative day care in England. Results of these studies are now in the public domain (1,2,3). The purpose here is to discuss some of the issues raised in this research and to comment further on some of the results.

Objectives: These were evaluative studies that subjected palliative care to the same kinds of analysis as are common in other areas of health care. In doing this there was a related objective of testing the suitability of different research methods for research on palliative care issues. The research also raised issues about the objectives of palliative care, and how research can help service providers to satisfy these issues.

Findings and issues raised: The research included interviews with patients. It was notable that almost all the patients who were invited to participate were keen to do so, and they were both more able and keener to do so than was estimated by care staff. The benefits of attending palliative day care included both more medical and more social features, and particular value was placed on the availability of easy access to skilled professionals as well as for interesting and stimulating activities. Patients were grateful for the efforts to provide diverse activities, but it was also clear that they did not share some of the agenda of the providers. They were able to identify services that were a priority, and equally those that were pleasant but unimportant. It was clear that patients had played little part in the formal design of the service packages, and if they had some different patterns would have occurred. The research had aimed to assess cost-effectiveness and found the tools performed badly. It was also found that it was more productive to focus on helping design content of palliative care than to assess its overall effectiveness.

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C21 DETERMINING QUALITY OF LIFE IN ALS/MND

O'BOYLE C

No abstract available.

C22 'AFFIRMING LIFE: PREPARING FOR DEATH': DUAL CHALLENGES IN ADDRESSING QUALITY OF LIFE FOR PEOPLE WITH MOTOR NEURON DISEASE

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A significant body of research exists examining factors correlated with Quality of Life (QoL) in motor neuron disease (1–4), much concluding that key determinants are factors that have a high subjective loading, including perceived quality of social support, spirituality and religiousness, emotional support and perceived caregiver distress.

Therapists working with people with motor neuron disease often have to acknowledge that changes in perspective are required from more traditional rehabilitation focused approaches from those which perhaps encourage the relinquishing, rather than achievement of new roles. As with many habits and roles in life, people have their own ways, their own standards and their own expectations of how they will want to fulfil them before handing them over. This presentation attempts to make explicit some of the concrete ways in which the research on Quality of Life can be used to guide professionals' interventions to support patients in the achievement and/or termination of roles, habits and valued occupations.

Working with concepts of 'pain', 'suffering' and 'death and dying' does not often seem an attractive replacement for concepts of 'achievement' 'reward' and 'improvement' which reflect the narratives of most rehabilitation professionals' working lives. In many respects this may demand a return, for professionals, to fundamental therapeutic skill rather than a focus on extensive bodies of evidence in traditional rehabilitation,

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510046905

and a shift from 'therapist-as-expert' to 'therapist-as-facilitator'.

There is an appropriate place for rehabilitation in palliative care however, designed around inter-professional working which is flexible, responsive, and realistic and aims to 'help patients gain opportunity, control, independence and dignity'(5).

Therapists are required to maintain a dual focus in assisting patients to maintain quality of life – on both affirming life and preparing for death. Not only does this involve the dual focus on patients' needs, but also considerable levels of emotional energy and therapeutic skill from the therapist. How these skills are learned and developed depend as much on skilled reflection as on formal education; supervision, formal and informal, should focus on priming more inexperienced staff to develop and awareness of key learning opportunities available to them from all members of the team.

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Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 21–23

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SESSION 4A CELLULAR TRANSPORT AND TRAFFICKING

C23 AXONAL TRANSPORT IN HEALTH AND DISEASE

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The trafficking and transport of vital cellular cargoes through axons is critical for correct neuronal function. Although many motors and cargoes have been identified, we do not understand the molecular basis for the regulation of their transport through axons. Evidence for an axonal transport defect in ALS was reported several years ago, and recently new data have emerged to support this as a disease mechanism. Impairment of axonal transport may not be restricted to ALS; it may play a role in a number of other neurodegenerative disorders. A better understanding of the biological processes that control motor proteins, the transport of their cargoes, and the cytoskeleton is therefore desirable.

New models of ALS and related disorders suggest that motor protein mutations are sufficient to disrupt transport and initiate pathogenesis in motor neurons. In addition, the hallmark pathology of protein accumulations found in mutant SOD1 transgenic mice and human ALS patients may be a cause or a consequence of disrupted axonal transport. Thus, there is evidence that deregulation of axonal transport contributes to the initiation, progression or pathology of ALS. It is therefore a challenge to determine the mechanisms downstream of mutant SOD1, which lead to transport defects.

C24 QUANTIFICATION OF AXONAL MITOCHONDRIAL TRANSPORT IN MOTOR NEURON DISEASE

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Amyotrophic lateral sclerosis (ALS) is a neurological disease characterized by progressive loss of motor neurons. About 10% of ALS cases are genetic, and mutations in the antioxidant enzyme Cu/Zn superoxide dismutase (SOD1) account for about 20% of these familial ALS cases. Axonal transport of membrane-bound organelles and the cytoskeleton by molecular motors is essential for neuronal development, maintenance, function, and survival. Defects in axonal transport have been reported in ALS and are hypothesized to contribute to disease initiation, progression, and pathology. Except in rare cases where ALS is linked to mutations in motor proteins, the nature and cause of ALS-related defects in axonal transport are poorly documented and understood. In addition, it has been suggested that mitochondrial dysfunction contributes to ALS pathology.

Using a semi-automated analysis routine, we have quantified all aspects of axonal transport of organelles from time-lapse recordings, namely, net transport, transport frequency, and velocity and persistence of movement. Net transport was measured by comparison of the start and end position per organelle whereas transport frequency was defined as anterograde or retrograde movements per organelle per second. These two parameters measure transport activity. The transport velocity and persistence represent characteristics of the molecular motors driving transport.

To investigate the role of axonal transport of mitochondria in ALS we quantified axonal transport of mitochondria in the mutant SOD1 model of ALS, focusing on three mutants G37R, G93A, and I113T. We found that expression of mutant SOD1 in cortical neurons enhanced net retrograde transport of mitochondria. This increase in retrograde transport correlated with higher frequency of retrograde transport. Interestingly the frequency of anterograde transport remained at control levels. The velocity and persistence of transport were unaffected, indicating the molecular motors driving transport retained normal functionality. Taken together, these results suggest that mutant SOD1 specifically enhances retrograde transport activity. Possible molecular mechanisms and implications for ALS will be discussed.

C25 ALS MUTANT SOD1 DISRUPTS BOTH FAST AND SLOW AXONAL TRANSPORT

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Background: The majority of neuronal proteins are synthesized within cell bodies and then transported to their appropriate locations. Some proteins are transported at fast rates (fast axonal transport) whereas others are transported at much slower rates (slow axonal transport). Disruption to slow axonal transport is an early pathogenic feature in a number of transgenic mouse models of ALS including those expressing mutant SOD1. However, the mechanisms by which mutant SOD1 damages axonal transport are not clear. We therefore examined movement of GFP-tagged cargoes in living neurons in the presence of either wild-type or mutant SOD1.

Objectives: The objectives were to gain insight into the mechanisms by which mutant SOD1 damages axonal transport.

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045122

Methods: Neurons were transfected with GFP- and/or DsRed-tagged versions of the amyloid precursor protein (APP) and either wild-type or mutant (A4V, G37R, G85R, G93A) SOD1. Movement of APP-GFP and GFP-SOD1 were monitored in living cells, and speeds, directions and pause-times determined.

Results: Movement of APP-GFP and GFP/DsRed-SOD1 through axons of cultured rat neurons were monitored. APP is known to move in the fast, whereas SOD1 moves in the slow, axonal transport compartment. Examination of APP-GFP and GFP/DsRed-SOD1 in neurons revealed that they were expressed as full-length proteins and that they were transported in different cargo complexes. We then examined the effect of expression of wild-type or mutant SOD1 on transport of APP-GFP. Mutant SOD1 significantly inhibited movement of APP-GFP in both anterograde and retrograde directions. This was due to slower speeds of movement of APP-GFP and also to an increase in the proportions of APP-GFP in the stationary vs. moving compartment. Likewise, mutant SOD1 transport was also disrupted and this was again due to slower velocities of movement and increased pausing in movement.

Discussion: We demonstrate that four different mutant SOD1 proteins all damage axonal transport. Damage is to cargoes moving by both fast and slow axonal transport, and is seen in both anterograde and retrograde directions. The slower speeds of movement seen in the presence of mutant SOD1 suggest that damage to motor function may be an aspect of toxicity.

C26 ALS2CL, A NOVEL ALS2 INTERACTING PROTEIN, MODULATES THE ALS2-MEDIATED ENDOSOMAL DYNAMICS

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Background: The loss-of-functional mutations in the *ALS2* gene account for a number of recessive motor neuron diseases (MNDs) including forms of amyotrophic lateral sclerosis, primary lateral sclerosis, and hereditary spastic paraplegia. The *ALS2* gene product, ALS2/alsin, is supposed to play an important role in maintenance and/or survival of motor neurons. Previous studies have demonstrated that ALS2 mediates the activation of Rab5 GTPase via its guanine nucleotide exchanging (GEF) activity, and modulates endosome dynamics in the cells. Recently, a novel *ALS2* homologous gene, ALS2 C-terminal like (*ALS2CL*), was identified. Thus, it can be hypothesized that ALS2CL modulates the ALS2-mediated cellular processes. However, the molecular and cellular functions of ALS2CL and its functional relationship with ALS2 are still unknown.

Objectives: To delineate the molecular and cellular functions of ALS2CL, and the functional relationship between ALS2 and ALS2CL in the cells.

Methods: We have investigated: 1) the expression and tissue distribution of ALS2CL by Western blot analysis using the ALS2CL-specific antibody; 2) the GEF activities associated with ALS2CL on a number of small GTPases using a biochemical assay; 3) the molecular interaction between ALS2 and ALS2CL using the yeast two-hybrid system and co-immunoprecipitation; 4) the subcellular distribution of ALS2CL, ALS2, and Rab5 in the cultured cells; and finally 5) the effect of co-expression of ALS2 and ALS2CL on endosome dynamics in the cells.

Results: ALS2CL was expressed in various mouse tissues with lower expression in central nervous system, and was particularly enriched in the detergent-insoluble cellular fractions. It exhibited a specific but a relatively weak Rab5-GEF activity with accompanying rather strong Rab5-binding properties. Using the yeast two-hybrid and co-immunoprecipitation, it has been shown that ALS2CL can directly interact with ALS2. In HeLa cells, co-expression of ALS2CL and Rab5A resulted in a unique tubulation phenotype of endosome compartments with significant colocalization of ALS2CL and Rab5A. Co-expression of ALS2CL and ALS2 revealed their co-localization in the vesicular compartments. Furthermore, overexpression of ALS2CL suppressed the enlargement of endosomes that was induced by the expression of a constitutive active form of ALS2.

Discussion and conclusions: Our results suggest that ALS2CL is a novel ALS2 binding protein and may play an important role in the ALS2-mediated membrane/endosome trafficking in the cells. Further characterization of these proteins in neuronal cells will clarify the implication of the ALS2CL-mediated functions in the pathological conditions in which expression of ALS2/alsin is lacking.

C27 NEURONAL EXPRESSION OF AN ALS-ASSOCIATED MUTANT DYNACTIN P150^{GLUED} IN MICE CAUSES MOTOR NEURON DISEASE

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Background: Impaired axonal transport has been implicated in a number of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). Recently, a missense mutation in a gene encoding dynactin (p150^{Glued}), a motor protein involved in retrograde axonal transport was linked to an autosomal dominant slowly progressive disease involving lower motor neurons (1,2).

Objectives: To examine the molecular mechanisms whereby mutant dynactin causes selective motor neuron loss.

Methods: We have generated transgenic mice overexpressing either the wild-type protein or the mutant (G59S) dynactin $p150^{Glued}$ driven by a neuron specific Thy1 promoter. Herein, we describe clinical phenotype, neuropathology and EMG analyses.

Results: To date, two independent mutant but not wild-type $p150^{Glued}$ transgenic lines of mice exhibit clinical and neuropathological features consistent with motor neuron disease. The mutant mice initially show spontaneous tremor that progresses with age, followed by gait abnormalities, poor grooming and hind limb weakness. Subsequently, mice lose weight and display hind limb muscle atrophy and severe paralysis. Electromyographic analysis of muscles discloses spontaneous fibrillations and spontaneous, positive sharp waves characteristic of denervation. Histological analyses of muscle showed denervation atrophy. In the spinal cord and brain stem, mutant mice show degeneration of motor neurons, characterized by large intracellular protein aggregates immunoreactive with antibodies to ubiquitin and to dynactin $p150^{Glued}$. Subsets of myelinated axons in ventral roots and peripheral nerve are swollen and some exhibit Wallerian degeneration. Cytoskeletal abnormalities include distinctive silver stained aggregates within the motor neurons. There appears to be a 40–60% loss of motor neurons at end stage of disease.

Discussion: These results indicate that neuronal expression of mutant dynactin $p150^{Glued}$ is sufficient to cause motor neuron disease and are consistent with the view that the disease arises from a toxic gain of function mechanism. We believe that this novel mouse model will prove to be a useful tool for examining the mechanisms of transport abnormalities underlying ALS and will be of value for the design of potential therapies.

Acknowledgement: This work was supported by Packard Center for ALS Research at JHU and NINDS.

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Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 24–26

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SESSION 4B NUTRITIONAL CARE AND FUNCTIONAL INDEPENDENCE

C28 TYPES OF THE OESOPHAGEAL BODY PERISTALSIS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS WITH DYSPHAGIA

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Background: The swallowing mechanism in ALS patients has not been systematically studied. As we have previously demonstrated, oesophageal manometry (OM) is an objective manometric method for detection and rehabilitation of oesophageal peristalsis failures by means of biofeedback training in ALS patients. We are continuing the research of the importance of OM in ALS patients with dysphagia.

Objective: To determine the types of the oesophageal body peristalsis in ALS patients with dysphagia.

Material and methods: We prospectively studied oesophageal body peristalsis, by using OM, in 25 consecutive ALS patients with dysphagia diagnosed in Krakow MND Centre (2004–2005), who fulfilled WFN Criteria (1998). All the patients underwent gastroscopy to exclude any anatomical and organic disorders of the oesophagus which would be contradictions for performing OM. The standard transnasal OM was performed in all of the patients, using a flexible catheter with three solid state transducers. The swallows were initiated with 5 to 10 ml of water (wet swallows) and saliva (dry swallows), and repeated at 30–sec intervals. The median pressures 5 cm below and within the upper oesophageal sphincter (UOS), the maximum pressures in the oesophageal body, the primary and secondary peristalsis of the oesophagus, the length of UOS, the velocity of the oesophageal waves propagation between UOS and 5 cm below it, as well as the time of the oesophageal contraction during the wet and dry swallows, were measured in all the ALS patients. The manometric parameters of the oesophagus were measured automatically, and visualized by the computer system. The tracings were analysed using Synectics software.

Results: We recognized three different types of oesophageal body peristalsis in ALS patients with dysphagia: 1) with increasing resting pressure value in the UOS >40 mmHg, with prolonged contractions in the oesophageal body >5 sec and the wave-like course of resting pressure in 12 out of 25 cases studied; 2) with low amplitude of peristaltic waves <25 mmHg without any signs of oesophageal motility disturbances in 10 out of 25 cases studied; 3) with no evidence of propulsive waves (i.e. primary and secondary peristalsis) in the whole oesophageal body and with successful swallowing ('gravity swallowing') in three out of 25 cases studied. The results will be presented in detail.

Conclusions: OM procedure allows us to distinguish the different types of the oesophageal body peristalsis in ALS patients with dysphagia. Interestingly, we demonstrated a few patients without any persistent oesophageal peristalsis function with successful swallowing ('flow peristalsis') and without clinical signs of aspiration. Therefore, we hypothesize that in these ALS cases the inhibiting non-adrenergic non-cholinergic (NANC) system is activated while the somatic cholinergic one is diminished.

C29 ASSESSING AND PREDICTING SUCCESSFUL TUBE PLACEMENT OUTCOMES IN ALS PATIENTS

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Background: A significant problem for ALS patients is maintaining adequate nutrition in the face of dysarthria, oromotor weakness, reduced upper extremity function and significant fatigue. Although bulbar patients are clearly at risk of malnutrition due to dysphagia, we have found that non-bulbar patients are at similar risk. Many patients opt for feeding tube placement to augment and eventually replace oral feeding. Despite promoting early tube placements while patients are in 'better' health, they often delay these decisions, which increases patient risk. This retrospective study reviews tube placement outcomes in 64 ALS outpatients seen by our interdisciplinary team during a five-year period.

Objectives: To determine at which point the risks outweigh the benefits of tube placement by reviewing outcomes against parameters of respiratory function, nutritional status and speech and swallowing deterioration. This information will help us provide informed opinions to patients with late stage ALS regarding the possible outcomes of delayed tube placements and guide patient decision-making around the timing of feeding tube placement.

Methods: A retrospective review of all consecutive feeding tube placements in an ALS outpatient clinic between January 2000 and 2005 was performed. Data were obtained from medical records describing respiratory function, nutritional status, and speech and swallowing function at time of tube placement.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045131

Results: A statistically significant association ($p=0.0058$) between nutritional status (% change in Usual Body Weight UBW (1)) and successful tube placement outcomes i.e. >1 month survival post- tube placement was found. The odds of a successful outcome in the subjects who were ⩾74% UBW were about 11 times as likely compared to the odds of a successful outcome in subjects with <74% UBW. No association was found between variables of respiratory status (FVC) ($p=1.000$), speech ($p=0.4243$) and swallowing ($p=0.4943$) (ALS Severity Score (ALSSS) (2)) outcomes. The number of deteriorated variables present per patient was not associated with success of outcomes.

Discussion and conclusions: The only variable of statistical significance was that of nutritional deterioration, i.e. reduction in usual body weight below 74%. Trends in data indicate a 100% success rate for outcomes that occurred with ALSSS for Speech and Swallowing greater than 7. These scores correspond to mild impairments, supporting early tube placement sometime before dysphagia impacts total oral intake. No association was found between tube placement outcomes and variables of respiratory, speech and swallowing function.

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C30 ENHANCING PEG TUBE PLACEMENT: A NEW DEVICE FOR MAINTAINING RESPIRATORY FUNCTION DURING ENDOSCOPY IN ALS PATIENTS

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Background: Percutaneous endoscopic gastrostomy (PEG) placement in patients with ALS has been shown to improve quality and duration of life by maintaining adequate hydration, nutrition and compliance with medications. Patients requiring PEG placement often suffer from significant respiratory insufficiency, increasing the risk of respiratory complications during endoscopic procedures and conscious sedation. Available alternative methods such as radiologically implanted gastrostomy (RIG) tube placement can be suboptimal due to a smaller lumen diameter and increased incidence of clogging. RIG tubes are also more easily pulled out accidentally, require radiation exposure, and can be an uncomfortable procedure for the patient. Optimizing PEG placement would involve a means of concurrently delivering adequate respiratory support in appropriate patients.

Objectives: To devise and implement a new device/technique which would provide adequate oxygenation/ventilation during endoscopic evaluation and intervention in order to initiate safer PEG tube placement.

Methods: A novel double channel face mask has been utilized that allows simultaneous endoscopy and ventilation/oxygenation. One port is utilized for the mechanical ventilation. A second port incorporates a fenestrated diaphragm which produces an airtight seal around the endoscope. This ensures a closed system, allowing for sufficient airway pressure to maintain adequate airway management. A respiratory technician is present and delivers mechanical ventilation using a pressure control mode to allow for minimal leaks and 100% FiO2. Patients are selected for use of the mask based on a combination of clinical judgment (the use of accessory respiratory muscles), significant dependence on BIPAP, and impaired vital capacity.

Results: To date, six patients have undergone PEG placement using the double channel face mask. No respiratory complications have occurred and none of the procedures had to be aborted. Oxygen saturation was maintained at ⩾95% throughout all procedures in this population with a fragile respiratory status. Adequate sedation was also used with complete post-procedure amnesia.

Discussion: PEG placement is essential in the management of the ALS population. Successful placement is often hampered by profound respiratory insufficiency significantly limiting endoscopic approaches and conscious sedation. This new technique facilitates the safe placement of a PEG ensuring minimal respiratory compromise, adequate sedation, and comfort for the patient.

C31 IMPROVING GASTROSTOMY CARE WITH SKIN LEVEL PRG

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Background: Percutaneous radiological gastrostomy (PRG) is an alternative to PEG insertion in MND/ALS patients. It appears to be safe and effective and allows NIPPV to be used during the procedure. Experience using the standard PRG pig-tail and balloon gastrostomy tubes revealed they were prone to leakage of gastric contents, blockage, tube failure and were easily displaced.

Objectives: After discussions with the interventional radiologist we decided to trial a new skin level PRG called Entristar and compare the results with those using the standard Wills-Oglesby™ pig-tail tube.

Methods: Patients requiring PRG were admitted to King's College Hospital. Retrospective data collection was performed including nutritional status, site of disease onset, infection, tube displacement and tube failure. Patients were followed up in the specialist clinic or by telephone contact.

Results: Thirty-five patients had the Wills-Oglesby™ tube, and The Entristar™ tube was inserted in 29 patients without complication. The incidence of infection was significantly lower in the Entristar™ group, ($p < 0.001$). The mean time to tube removal in the Entristar group was 223 days (SD 147; range 71–494 days) due to 'buried bumper syndrome'.

Discussion: The Entristar™ skin level gastrostomy is a robust tube and is associated with reductions in infection, tube failure and blockage leading to improved patient care.

C32 DOES REHABILITATION MAKE SENSE IN MOTOR NEURON DISEASE?

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While someone with an acquired injury such as a stroke clearly requires rehabilitation, progressive conditions such as motor neuron disease (MND) seem more problematic. In recent years, however, there has been growing awareness that the principles of rehabilitation are relevant to all kinds of disability. The WHO International Classification of Functioning, Disability and Health focuses on participation, the fulfilment of roles that are either essential to the individual or the result of autonomous choice. All human beings have such roles, not least those who are in the late stages of progressive diseases. One modern definition of rehabilitation is 'the use of all means to minimize the impact of disabling conditions and to assist disabled people to achieve their desired level of autonomy and participation'. By this definition, rehabilitation is relevant to people at all stages of MND. For one individual at one stage of the condition the rehabilitation agenda might be very similar to that of a middle-aged person who has been injured at work, with an emphasis on physical independence, mobility and job retention. For someone with more severe or more rapidly progressive MND, the aim might be autonomy rather than independence, and the pivotal role might be family participation rather than employment. In either case, the best outcomes are achieved within a rehabilitation framework where specific, measurable goals are related to the person's aims – always with due regard for the needs of carers and others.

Neurological rehabilitation services in Derby include an inpatient element which is occasionally relevant for people with MND, but the core of the service is community-orientated. The service is underpinned by channels of communication, first with the nearby MND Association Care and Research Centre, with hospital-based resources such as the nutrition team, with specialist social services-funded Care Managers for neurological clients and with other community professionals. Inter-professional communication is sustained by regular meetings of a Rehabilitation Communication Group based in each Primary Care Trust area. People with complex progressive neurological conditions who consent to be included in the system are discussed. This allows new problems to be understood more rapidly, and supports the work of those professionals who are directly involved. Many of the medical as well as non-medical assessment and interventions are home-based.

In this talk I will discuss some of the barriers to effective rehabilitation-orientated services, and some of the possible criteria for success. Good rehabilitation must be adequate but not excessive, and neither too early nor too late. Patients with MND and carers can be either neglected or overwhelmed by services, expectations can be falsely raised, and delays can be critical. Success will always depend on the extent to which autonomy and participation are maximized at every stage of life.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 27–29

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SESSION 6A INFLAMMATION

C33 ACTIVATED MICROGLIA IN ALS: A DOUBLE-EDGED SWORD

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Background: Recent studies of ALS suggest that motor neuron death is non-cell autonomous, with cell injury possibly mediated by interactions with non-neuronal cells such as microglia. Our recent *in vitro* data demonstrated that activated microglia release free radicals, including nitric oxide (NO) and superoxide ($O_2^{\bullet\,-}$), and initiate motor neuron (MN) injury by a glutamate-mediated effect. Microglia can also release neurotrophic factors, including IGF-1, as well as free radicals. A key question is whether microglia can be induced to protect against motor neuron injury *in vitro*, and whether mSOD1 microglia are less neuroprotective than wild-type microglia.

Objective: To determine whether microglia can be neuroprotective, and if so, by what mechanisms.

Methods: Primary motor neuron cultures were prepared from embryonic rat spinal cords. Microglial cultures were prepared from Sprague Dawley rats and mSOD1 and wild-type littermate mice. The anti-inflammatory cytokines, IL-4 or IL-10, were applied to cultures, followed by LPS. The production of NO was estimated in the culture media and iNOS expression was measured by Western blots. IGF-1 mRNA was detected by RT-PCR, and protein levels of IGF-1 and IL-4 were measured by ELISA. MN survival was quantified by direct counting of all p75 positive live cells displaying intact neurites longer than three cell diameters.

Results: IL-4 and IL-10 significantly reversed motor neuron injury induced by microglia activated by LPS. The increased motor neuron survival correlated directly with the decrease in NO production. IL-4 and IL-10 suppressed NO release by down-regulating microglial iNOS. Although IL-4 and IL-10 enhanced IGF-1 protein levels in motor neuron and resting microglia co-cultures (in the absence of LPS), IL-4 and IL-10 had minimal effects on IGF-1 protein levels (<10 pg/ml) in motor neuron and microglia co-cultures treated with LPS. IGF-1 at 2–50 ng/ml did not protect against the cytotoxic effects of peroxynitrite and glutamate on motor neuron cultures. Thus, IGF-1 does not explain the neuroprotective effects of IL-4. mSOD1 microglia expressed higher NO levels, lower levels of iNOS, and lower levels of IGF-1, and were less neuroprotective than wild-type microglia.

Conclusions: The anti-inflammatory cytokines, IL-4 and IL-10, protect against motor neuron toxicity induced by activated microglia. This neuroprotection is mediated by down-regulation of iNOS expression and lowered production of NO. It does not appear to depend upon IGF-1 production or release. These anti-inflammatory cytokines may have possible therapeutic value in ALS primarily by their ability to down-regulate the production and release of free radicals. Furthermore mSOD1 microglia appear to have significantly less neuroprotective capacity.

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045140

C34 MICROGLIAL ACTIVATION IN CX3CR1 NULL-SOD1(G93A) ALS MICE WORSENS DISEASE PHENOTYPE

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Background: Microglial mediated inflammation has been implicated in the loss of motor neurons (MNs) of amyotrophic lateral sclerosis (ALS). The fractalkine receptor, CX3CR1, is localized to microglia and suppresses their activation via its ligand, fractalkine. The transgenic (Tg) mouse model of ALS expressing the G93A-mutated human SOD1 gene ($SOD1^{G93A}$), exhibits age-dependent degeneration of MNs, limb weakness, and early death.

Objectives: To determine if $TgSOD1^{G93A}$ mice without fractalkine receptor, i.e. null for the CX3CR1 gene ($CX3CR1^{-/-}$), experience more microglial activation, greater MN loss and limb weakness, and shorter survival than Tg mice with at least one copy of the gene ($CX3CR1^{-/+}$).

Methods: We established a colony of mice that were $TgSOD1^{G93A}$-$CX3CR1^{-/-}$, $TgSOD1^{G93A}$-$CX3CR1^{+/-}$, and non-$TgSOD1^{G93A}$-$CX3CR1^{-/-}$ (as unaffected littermate controls), and performed serial behavioral and survival assessment, with histological analysis of terminal mice at 130–140 days.

Results: Microglial reaction was increased in the lumbar spinal cords of $TgSOD1^{G93A}$-$CX3CR1^{-/-}$ mice compared with either $TgSOD1^{G93A}$-$CX3CR1^{+/-}$, or non-$TgSOD1^{G93A}$-$CX3CR1^{-/-}$ animals. There were significantly fewer Nissl-stained lumbar cord MNs in $TgSOD1^{G93A}$-$CX3CR1^{-/-}$ mice (mean % reduction of MNs $=58.6\pm4.6\%$; $n=5$) compared with $TgSOD1^{G93A}$-$CX3CR1^{+/-}$ mice ($42.2\pm5.5\%$; $n=5$; $p=0.03$), using non-$TgSOD1^{G93A}$-$CX3CR1^{-/-}$ littermates ($n=5$) as a reference for both. After 7–9 weeks of age, hindlimb strength was lower in $TgSOD1^{G93A}$ mice compared to non-$TgSOD1^{G93A}$ littermates. From 15 to 20 weeks, when weakness became progressive, the rate of decline in Tg mice was much faster in the $CX3CR1^{-/-}$ group than in the $CX3CR1^{+/-}$ group ($p<0.01$, random

coefficient regression analysis). Furthermore, this more rapid decline resulted from male CX3CR1$^{-/-}$ mice (slope=-19.92 ± 2.1, SE, $n=5$) rather than female CX3CR1$^{-/-}$ mice (slope=-8.41 ± 2.4, SE, $n=7$). Random coefficient regression analysis also demonstrated a significant mouse-group by gender by age interaction that indicated faster loss of body weight ($p<0.01$) and forelimb grip strength ($p=0.02$) for male TgSOD1^{G93A}-CX3CR1$^{-/-}$ mice compared to all other TgSOD1^{G93A} mice. Kaplan-Meier analysis revealed survival was shorter by almost two weeks in TgSOD1^{G93A}-CX3CR1$^{-/-}$ mice (129 days) compared to TgSOD1^{G93A}-CX3CR1$^{+/-}$ mice (141 days, $p=0.01$).

Conclusion: Absence of the CX3CR1 in TgSOD1^{G93A} mice results in pronounced microglial reaction, greater MN loss, worsened neurobehavioral outcomes, and shorter survival, with the effect preferentially expressed in males. Future studies will elucidate the downstream mediators of microglial injury in an effort to identify novel therapeutic targets in ALS.

C35 TRANSCRIPTIONAL REGULATION OF INFLAMMATORY PROCESSES IN AMYOTROPHIC LATERAL SCLEROSIS: EXPRESSION OF CCAAT/ENHANCER BINDING PROTEIN (C/EBP) ß, SUPPRESSORS OF CYTOKINE SIGNALLING (SOCS) AND INTERFERON REGULATORY FACTOR-1 (IRF-1)

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Background: Elevations of numerous proinflammatory cytokines and chemokines in the central nervous system of amyotrophic lateral sclerosis (ALS) patients as well as in an animal model of familial ALS (SOD-transgenic mice) indicate that inflammatory mechanisms may contribute to motor neuron death in this disease. However, little is known about exact functions, interactions, and the regulation of these molecules in ALS pathogenesis.

Objectives: The expression of several molecules which may be involved in the regulation of inflammatory processes was investigated in spinal cords of ALS patients and SOD-transgenic mice.

Methods: The microarray technique was used to study transcriptional changes of these molecules. Furthermore, we used immunoblotting and immunohistochemistry to investigate the amount of the corresponding protein and to identify the cell species in which these proteins are expressed.

Results: Microarray analysis revealed enhanced expression of CCAAT/enhancer-binding protein-ß (C/EBP ß), interferon regulatory factor-1 (IRF-1) and suppressor of cytokine signalling 1 (SOCS1), as well as a reduced expression of SOCS3 mRNA in the spinal cord of ALS patients and SOD-transgenic mice. These results were confirmed by immunoblotting. Furthermore, several transcriptional targets of C/EBP ß, including interleukin 6 (IL-6) were up-regulated in the same specimens. Immunohistochemistry showed that C/EBP ß, IRF-1, and SOCS proteins were mainly expressed in microglial and astroglial cells of the spinal cord ventral horn.

Discussion and conclusions: C/EBP ß is a transcription factor that couples extracellular signals to various intracellular processes but also participates in inflammatory processes. IL-6 is not only a transcriptional target, but also a strong inductor of C/EBP ß expression. Elevated levels of these molecules may be part of a feed-forward mechanism causing the spreading of the inflammation. The SOCS family comprises proteins induced on cytokine stimulation, which blocks further signalling in a feedback loop. SOCS 1 is the most potent inhibitor of cytokine signalling. Therefore, the elevated expression of SOCS1 in ALS may be an attempt to limit inflammatory processes in ALS. SOCS3 is a negative regulator of IL-6. SOCS 3 deficiency, leading to sustained IL-6 activation, has been associated with the development of chronic inflammatory diseases. IRF-1 activates the expression of various downstream targets; for example, it has been associated with the induction of inducible nitric oxide synthase (iNOS). The astrogliosis and microgliosis observed in ALS are associated with altered levels of C/EBP ß, SOCS proteins and IRF-1. These molecules may act as regulators of inflammatory signalling in this disease.

C36 DEFINITION OF A TRANSCRIPTIONAL SIGNATURE OF PERIPHERAL MYELOID CELLS ASSOCIATED WITH SEVERE NEURODEGENERATIVE DISEASE

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Background: Amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), macular degeneration (MDgn) and HIV associated dementia are all characterized by elevated levels of activated (CD14/16++) macrophages in peripheral blood. This common phenotype suggests that these disparate diseases may share common mechanistic features. One indication of a common mechanism would be similar alterations in gene expression patterns relative to healthy controls.

Objective: To evaluate individuals with elevated levels of activated macrophages in their peripheral blood for common gene expression signatures.

Methods: Blood samples were obtained from individuals with ALS ($n=32$), AD ($n=9$), MDgn ($n=22$), HIV infection ($n=5$), and healthy controls ($n=20$) after obtaining informed consent. Mononuclear cells were isolated by Percoll gradient centrifugation. The cells were

then cultured overnight at 37°C under non-adherent conditions, collected, lysed, and total RNA prepared. RNA expression data were obtained using an Affymetrix scanner and protocols. Selected genes identified by the microarray data were selected for quantitative RT-PCR analysis using a Light-Cycler. Genes were evaluated for coordinated transcription by determining the Pearson correlation coefficient (R) for each probe set with other probes expressed in ALS patients (~23,000). Pairs of probes with an $R > 0.7$ ($p < 0.0001$) were said to exhibit correlated transcription.

Results: Samples from individuals with ALS (30 of 32), AD (9 of 9) and HIV infected individuals (4 of 5) all had a group of ~500 genes that had R values of 0.7 or more with the majority of other members of the group. The genes in the signature were expressed at low levels and did not exhibit correlated transcription in healthy age-matched controls. A significant fraction of the signature included genes known to be expressed in myeloid cells. Another prevalent group in the signature was genes associated with the α/β interferon-mediated antiviral response. Other signature genes included genes associated with the innate immune response and regulation of apoptosis. Individuals with MDgn did not exhibit significant up-regulation and correlated transcription of interferon response genes but did show elevation of some myeloid cell genes.

Conclusions: Peripheral blood cells from HIV infected individuals, ALS patients, and AD patients have a common transcriptional signature that is not seen in controls or individuals with MDgn. Although detection of a strong α/β interferon-mediated antiviral response would be expected in HIV infected individuals, its presence in individuals with ALS or AD is surprising and suggests that one feature of the chronic inflammation seen in ALS and AD is an unregulated or inappropriate antiviral response.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 30–32

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SESSION 6B CLINICAL PHENOTYPES

C37 REGIONAL DIFFERENCES BETWEEN LONG AND SHORT SURVIVORS IN ALS

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Background: Prognostic factors known to predict poor survival in ALS include older age, bulbar onset, reduced forced vital capacity, low scores on ALSFR-S, and short time period from symptom onset to diagnosis. In contrast, predictors of long survival are not well-characterized. Discussion of longer survival has generally been limited to reports of specific regional presentations, such as in brachial amyotrophic diplegia, where long-term survival can occur.

Objectives: To characterize features of long and short survivors in ALS using a large North American patient database.

Methods: The ALS Patient Care Database is the largest prospectively designed, clinically confirmed database of patients with ALS. It was created, in part, to measure outcomes in this disorder. For this study, we defined short survival in ALS as death within one year of symptom onset and long survival as death more than five years after symptom onset (without requiring ventilator support) or patients who are still alive, without ventilator support more than five years after onset. We queried the database to determine clinical features as close to the time of presentation as possible. Thus, we excluded long survivors who did not have at least one available data form from within two years after disease onset. We compared the clinical features of short survivors (SS) and long survivors (LS) and characterized key features of the long survivors.

Results: Of 5877 patients in the database, 1809 were known to have died as of the index date. Approximately 6% (101 patients) had survived for less than one year of onset and 18% more than five years. After the addition of still-surviving patients and exclusions, there were 86 long survivors (5.1–8.8 years). LS were more likely to have possible ALS by El Escorial criteria (LS: 15.4%, SS: 2.2%). Among LS, difficulty with climbing stairs was the most common functional abnormality (ALSFR-S), followed by difficulty walking and dressing. In contrast, more than 90% of the longest survivors reported little or no difficulty using BiPAP and swallowing. Short survivors were older (SS: 66.5, LS 54.6 years); had decreased FVC (SS: 56% of predicted, LS: 87%); and had lower ALSFR-S scores (SS: 26.5/40, LS 33/40). No differences were found in gender, chance of having only UMN symptoms, or having only LMN symptoms.

Conclusions: A new finding of this study is that among LS, difficulty climbing stairs and walking, along with consistently normal bulbar function and swallowing may indicate relatively isolated lower limb involvement. The high frequency of possible ALS in this group also points to a favourable prognosis for isolated regional presentations. Our findings also confirm earlier studies showing respiratory dysfunction, low ALSFR-S scores, and older age is associated with short survival. Prospective studies will determine how strongly these findings predict long survival in other patient cohorts. Future studies on prognosis may benefit from a focus on the rate of spread between regions in addition to the rate of progression.

C38 KENNEDY'S DISEASE: A CLINICAL AND FUNCTIONAL STUDY IN 44 PATIENTS

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Background: Kennnedy's disease is a lower motor neuron disease which is recessively inherited, in male patients, due to an abnormal expansion of CAG repeat in the first exon of the androgen receptor gene located on the X chromosome. Since the description of bulbo-spinal muscular atrophy in 1968 by Kennedy et al., the clinical hallmarks of this slowly progressing sensori-motor neuronopathy have been better characterized. However, little is known about disease onset modalities, functional status, rate of increasing weakness and life expectancy.

Objectives: To present clinical, functional, biological and neurophysiological data of 44 patients with Kennedy's disease (KD).

Methods: Forty-four patients seen in the Paris ALS centre have been extensively studied. They underwent neurological examination, functional rating, neuropsychological testing, biological and electrophysiological studies and molecular analysis. Statistical analysis was performed to look for relationships between time of onset, muscle function decline and size of CAG repeat.

Results: Mean age at the time of diagnosis was 56.8 years. The mean age at onset was 45.4 years. The main symptoms leading the patient to see their physician were lower limb muscle weakness and cramps; 74.2% of

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045159

patients had abnormal sensory nerve potentials. No correlation was observed between the age of onset, disease duration, and the size of the polyglutamine tract. The functional rating scales routinely used in ALS therapeutic trials such as the ALSFR-S were not appropriate in this disease because of the slow rate of decline in muscle function. Other scales used in multiple sclerosis have been used to monitor the progression of the disease.

Discussion and conclusions: KD is a rare, slowly progressive, motor neuron disease whose natural history remains unclear. Collecting data on muscle function decline is a first step in disease understanding and will help to design appropriate therapeutic trials.

C39 EARLY-ONSET ALS WITH 'SPASTIC PHENOTYPE': A DISTINCT ENTITY?

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Background and objectives: Although the great majority of ALS patients disclose a stereotyped clinical picture, noticeable heterogeneity exists regarding the age of onset, initial clinical manifestations and outcome. Most studies show that the age-specific incidence in sporadic ALS is very low in the third and forth decades and then markedly increases reaching a peak between 55 and 75 years. ALS occurs predominantly in males, with a male to female ratio of 1.4–1.7, but in ALS patients with onset of the disease before the age of 40 years this ratio is strikingly higher. Age of onset represents an important prognostic factor, survival being significantly longer in those patients with onset age less than 50 years compared to those with onset age in the sixth-eighth decades. Better prognosis in younger patients is explained on the basis of greater neuronal reserve with respect to older patients. There are very few reports in the literature concerning early-onset ALS. The aim of our study was to investigate the clinical features and the long-term follow-up of a group of ALS patients with an onset before the age of 40 years in order to establish whether early-onset represents a distinctive feature of an ALS subtype.

Methods: Three hundred and eighty-six ALS patients were observed in our Neurological Institute from 1987 to 2005. Forty-five ALS patients (11%) had an onset of the disease before the age of 40 years and were included in the study. These patients were followed for a mean period of 52.4 months (range 7–146 months).

Results: The age of onset ranged from 22 to 40 years. There were 37 males and eight females with a male to female ratio of 4.6:1. Seventeen of the 45 patients (38%) had the 'classic' clinical pattern with predominant lower motor neuron (LMN) signs and moderate pyramidal signs. The male to female ratio was 18:1. Eight patients died or underwent tracheostomy after a mean period of 47.2 months (range 18–106 months) from the onset. Nine patients are alive with a mean duration of disease of 34 months (range 7–91 months).

The remaining 28 patients (62%) disclosed a quite distinct clinical picture characterized, in the initial phases of the disease, by the following features: 1) Pyramidal signs were a striking feature, with a marked spastic paraparesis, brisk reflexes, pseudobulbar speech; 2) LMN signs (weakness, atrophy and fasciculations) were noticeable in the forearms and hands on both sides, but were scanty in the proximal upper limb muscles and in the lower limbs; 3) Respiratory muscles were involved late in the course of the disease. The male to female ratio was 13:1. All patients showed a clearly progressive course: 12 patients died or underwent tracheostomy after a mean of 53 months (range 14–74) from the onset. Sixteen patients are still alive with a mean duration of disease of 64.8 months (range 9–146 months). In the late stages of the disease LMN signs were clearly present in all four limbs and in the tongue.

Conclusions: Patients with young-onset ALS show two different phenotypes. In a first group of patients' clinical manifestations, male/female ratio and outcome are similar to those observed in the 'classic' form. A second group of patients has a stereotyped clinical picture, which appears to be an intermediate form between primary lateral sclerosis and classic ALS. These patients represent the majority of early-onset ALS (62%) and disclose an unusual male predominance. The duration of the disease is significantly longer than that observed in ALS with an onset after 40 years. Based on these data the hypothesis that young-onset ALS with the 'spastic phenotype' is a distinct entity should be taken in account.

C40 FURTHER INSIGHTS INTO HSP AND SPASTIN: SEVERE COMPLICATED PHENOTYPES, DOUBLE MUTATIONS AND EVIDENCE OF LOWER MOTOR NEURON DYSFUNCTION

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Background: Mutations in the spastin gene are the commonest cause of hereditary spastic paraparesis (HSP) accounting for approximately 40% of autosomal dominant cases. The phenotype associated with HSP due to mutation in the spastin gene (*SPG4*) tends to be of pure HSP. At least 107 different mutations have been identified scattered along the length of spastin initially suggesting haploinsufficiency may be the pathogenic mechanism. However, recent work in cell models has suggested that in some cases an abnormal gain of function mechanism may occur.

Objectives: To characterize SPG4 genetically and phenotypically.

Methods: Sheffield offers an international screening service for mutations in the spastin gene. Patients were

identified who had tested positive for spastin mutations using a direct sequencing approach of all exons. Clinical details were readily available for those seen in the South Yorkshire region who had been assessed by PJS or CJM. Clinical details for patients from other centres were collected by use of a proforma sent to the referring neurologist.

Results: In 61 patients we have identified 47 different spastin mutations of which 25 are novel. Twenty missense, three nonsense, 12 frameshift and nine splicing changes were identified. These mutations were scattered throughout the gene with a particular hot spot in exon 1. Three patients were identified with two separate mutations within the spastin gene. The phenotype in the majority of patients was of pure HSP. However in two individuals the phenotype stood out as being particularly severe and unusual. In one, progressive bulbar dysfunction and respiratory insufficiency requiring overnight non- invasive ventilation complicated a severe spastic quadraparesis. In a second individual severe contractures, scoliosis and bulbar dysfunction complicated a spastic quadraparesis. A further observation in three individuals was the presence of denervation on neurophysiological assessment indicating lower motor neuron involvement.

Discussion: These findings add to the number of spastin mutations identified and demonstrate the importance of screening the whole gene given the possibility of double mutations. The hot spot for spastin mutation identified in exon 1 suggests an important functional role for this region of spastin which requires further investigation. The identification of the complicated phenotypes has important implications for patients in whom spastin screening should be considered. The presence of lower motor neuron dysfunction in a group of patients supports pathological evidence of lower motor neuron involvement, indicating that the cellular dysfunction in SPG4 extends beyond the axonal projections of upper motor neurons and ascending sensory pathways.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 33–35

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SESSION 7A PROTEIN FOLDING AND DEGRADATION DEFECTS

C41 FUNCTIONS OF THE PROTEASOME IN CELL REGULATION AND NEUROMUSCULAR DISEASE

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Most proteins in mammalian cells are degraded by the ubiquitin-proteasome pathway, where protein substrates are linked to ubiquitin molecules by one of the cell's many ubiquitin ligases (E3s). This modification marks the protein for rapid degradation by the 26S proteasome. Much has been learned about this large complex, which uses ATP to unfold proteins and to inject them into its 20S core proteasome where they are digested to small peptides. An important function of this system is to selectively destroy abnormally folded proteins as they unfortunately accumulate in various neurodegenerative diseases such as ALS. Protein inclusions in the affected neurons contain ubiquitin and proteasome. This observation and a number of studies suggest a failure of the degradation process (e.g. especially in HD and PD).

With denervation or disuse, in fasting, and in many systemic diseases (e.g. cancer, sepsis, diabetes, excessive glucocorticoids), muscles atrophy due to a general activation of the ubiquitin-proteasome pathway in muscles. The atrophying muscles show a common pattern of changes in expression of specific genes (which we term 'atrogenes'). The two proteins induced most dramatically are muscle-specific ubiquitin ligases, atrogin-1 and MuRF-1, which trigger the atrophy process and the accelerated proteolysis. Their expression is normally inhibited by IGF-1 and insulin, but rises in atrophying muscles due to activation of the transcription factor, Foxo3, which leads to excessive proteolysis and.

Much has been learned about the functions of this system by the use of inhibitors of the proteasome that enter cells and inhibit intracellular proteolysis. Blocking proteasome function eventually induces apoptosis, especially in cancer cells. One such inhibitor (Velcade PS341) has been approved by the FDA for treatment of multiple myeloma, but it is now in trials against diverse cancers.

Although most peptides released by proteasomes are rapidly digested to amino acids, some are transported through the ER to the cell surface, where they are presented to the immune system on MHC Class I molecules. This process enables circulating cytotoxic T-cells to screen for and eliminate virally infected cells and cancers.

C42 ROLE OF DYSFUNCTION OF THE UBIQUITIN PROTEASOME PATHWAY IN THE MECHANISMS OF MOTOR NEURON DEGENERATION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Accumulation of misfolded and ubiquitinated proteins in degenerating cells is a hallmark of both ALS patients and a murine model of the disease (SOD1G93A mice). The ubiquitin-proteasome pathway (UPP) is the main proteolytic system in eukaryotic cells. Increasing evidence suggests a link between UPP impairment and neurodegenerative diseases.

Objective: The aim of the study was to investigate the possible role of UPP in the pathogenetic mechanisms of ALS in SOD1G93A mice.

Methods: Experiments were conducted on SOD1G93A transgenic mice at various stages of disease progression; non-transgenic littermates were used as control. Biochemical quantification of proteasome activity was conducted by measuring the fluorescence of 7-amido-4-methylcoumarin (AMC) generated from cleavage of specific peptide-AMC linked substrates in tissue homogenates. The levels of inducible and constitutive proteasome subunits were analysed using Western blots. Immunohistochemical evaluation of 20S proteasome and ubiquitin levels and distribution was carried out on paraformaldehyde-fixed spinal cord cryosections using diaminobenzidine or immunofluorescent staining.

To evaluate the UPP activity *in vivo* and at the cellular level, SOD1G93A mice were cross-bred with a transgenic mouse model (GFP reporter mice) with constitutive and ubiquitous expression of Ub^{G76V}-GFP reporter for proteasomal degradation; the functional status of the UPP was analysed in double transgenic mice (GFP/SOD1G93A) by the levels of the reporter substrate.

Results: Biochemical assays of spinal cord tissue did not reveal any change of proteasome activity in the spinal cord of SOD1G93A mice compared to non-transgenic

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045168

littermates. However, we observed a significant reduction in constitutive catalytic subunits of the proteasome and increase in their inducible counterparts at an advanced stage of the disease. We also detected a selective decrease of 20S proteasome levels and an increase of ubiquitin signal in the spinal motor neuron during disease progression. Immunohistochemical analysis GFP reporter/SOD1G93A mice revealed accumulation of the reporter for proteasome activity in some neurons of the lumbar spinal cord at the end stage of the disease.

Conclusions: Although no changes of proteasome activity were found in the spinal cord homogenates of SOD1G93A mice during disease progression, we revealed a reduction of 20S constitutive proteasome and the concomitant increase of immunoproteasome. In fact, analyses on GFP/SOD1G93A mice revealed decrease of UPP function in some neurons of lumbar spinal cord.

Acknowledgement
This work was supported by Telethon, Italian Ministry of Health, MIUR.

C43 MECHANISMS BY WHICH EXPRESSION OF MUTANT SOD1 IMPAIRS PROTEASOME FUNCTION IN VULNERABLE SPINAL CORD TISSUE

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Background: In experimental models familial ALS caused by mutations in the Cu/Zn-superoxide dismutase (SOD1) gene, death of motor neurons is preceded by aggregation of mutant SOD1 and formation of cytoplasmic inclusions. Proteinaceous inclusions are also a hallmark of sporadic ALS and other neurodegenerative disorders, and their occurrence suggests overload of proteolytic pathways. We previously reported impaired proteasomal function in the lumbar, but not thoracic, spinal cord of pre-symptomatic G93A SOD1 transgenic mice relative to non-transgenic littermates and wild-type (WT) SOD1 transgenic mice (1). This compromise of proteasome function was compounded by reduction in the level of 20Sα subunits in lumbar spinal motor neurons despite maintenance of levels of 20Sα proteasomal subunits in the tissue as a whole. A subsequent study reported decrease in constitutive β5 and increase in β5i in symptomatic mice (2).

Objectives: To further investigate the mechanism of proteasome dysfunction by examining expression of other proteasomal subunits and proteasome assembly in tissues of G93A SOD1 transgenic mice compared to non-transgenic littermates and WT SOD1 transgenic mice, and in tissues from ALS patients.

Methods: Expression of proteasomal subunits was examined by Western blotting of homogenized lumbar and thoracic spinal cord and expressed relative to actin. Composition and assembly of proteasomes is examined by 2-D Blue Native/SDS-PAGE.

Results: Levels of the constitutive β5 subunit, responsible for the chymotryptic-like activity of the proteasome, and the structural β3 subunit of the 20S proteasome were significantly reduced specifically in the lumbar region of the spinal cord of G93ASOD1 transgenic mice by 45 days of age. The reduction was dramatic by 75 days of age. Inducible β5i immunoproteasome subunit and regulatory components (19S6b and 11Sα) of the 20S proteasome remained unchanged at these ages.

Conclusions: The results provide additional evidence that proteasome dysfunction contributes significantly to pathogenesis of motor neuron disease. The early pre-symptomatic reduction in constitutive β5 subunits in lumbar spinal cord is consistent with reduction in chymotrypsin-like catalytic activity. The pronounced differences in expression of various proteasomal subunits in tissue vulnerable to the disease process indicates more complex changes than substitution of immunoproteasome subunits and suggests altered assembly of proteasomes and/or post-translational modification of subunits. This is being resolved by 2-D blue native/SDS-PAGE.

Acknowledgement
This work was supported by CIHR-ALS Society of Canada-MDAC neuromuscular partnership and ALS Association USA.

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C44 THE ROLE OF ENDOPLASMIC RETICULUM (ER) STRESS AND THE UNFOLDED PROTEIN RESPONSE (UPR) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Mutant SOD1 misfolded aggregates are a common feature of spinal cords from both human ALS patients and transgenic rodent models. Increasing evidence suggests that disturbances in the neuronal ER occur as an early event in the pathway to clinical ALS. It is known that in the presence of misfolded proteins in the lumen, the capacity of the ER to maintain protein folding can quickly become overwhelmed and the UPR may be initiated. This involves the up-regulation of a number of key chaperone and other proteins and the inhibition of further protein synthesis. If not sufficiently inhibited, the

ER stress may result in cell death by apoptosis regulated by caspase-12.

Objectives: The purpose of this study was to examine spinal cord tissue in the G93A mouse and rat models of ALS, and in NSC34 cells transfected with WT and mutant SOD1 constructs, for the presence of UPR/ER stress proteins.

Methods: Proteomic analysis was carried out to look for differentially expressed proteins using spinal cord tissue from two male transgenic SOD1 rats and one male normal control at p60. 2-DE gel electrophoresis was performed and differentially expressed spots were excised from the gel, digested with Trypsin for 16 h and subjected to MALDI-TOF mass spectrometry. Western blotting was performed to verify these findings and to look for the differential regulation of other proteins.

Results: Proteomic analysis has revealed that Hsp60, protein disulphide isomerase and Grp58 were significantly up-regulated in SOD1 rats and mice. Western blotting has also revealed up-regulation of BiP, ATF6α, CHOP, and caspase-12, the latter two proteins being thought to initiate entry into the apoptotic pathway. This is currently being investigated further.

Conclusion: These findings suggest that the UPR is initiated in ALS which may contribute to the initiation of the apoptotic pathway.

C45 MONOMERIC/MISFOLDED SOD1 DETECTED IN MOUSE MODELS OF ALS USING A DESIGNED ANTIBODY

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Background: Like other neurodegenerative diseases, protein aggregation is an important feature of the disease pathology. Inclusion bodies that are immunoreactive for SOD1 are present in both human cases of SOD1-ALS and in animal ALS models that express human mutant SOD1 on a normal mouse genetic background. Toxicity of these cytoplasmic aggregates is thought to arise from aberrant interactions with the protein-folding chaperone system or from inhibition of proteasomes. Toxicity has also been proposed to result from aberrant interactions with mitochondrial proteins such as Tom20 or Bcl-2 because SOD1 has been detected in mitochondria from the spinal cord and brain, and mitochondrial vacuolization is an early event in ALS models. These interactions constitute a gain-of-function since interactions with these proteins have been unreported under normal conditions. SOD1 must, by inference, expose novel sites of interaction, but this has not been demonstrated *in vivo*.

Objectives: To create an antibody that selectively recognizes misfolded SOD1, and to examine whether misfolded SOD1 exists *in vivo*.

Methods: We have previously shown that mutant SOD1 is more aggregation prone than wild-type SOD1 *in vitro* and both go through a monomeric intermediate prior to aggregation (1). We identified an epitope that is exposed only in monomeric SOD1 and not in native dimeric SOD1. We then generated an antibody directed against this epitope and verified that it reacts only with monomeric/misfolded SOD1. Misfolding of SOD1 in the G93A and G37R SOD1 mutant mice was then probed using this antibody.

Results and discussion: Monomeric/misfolded SOD1 was found in both G93A and G37R SOD1 mutant expressing mice, but not in non-transgenic littermates. This shows directly that SOD1-ALS is a misfolding disease. Misfolded SOD1 localizes primarily to motor neurons. Misfolded SOD1 accumulates with age, appearing before onset of rear-leg weakness. Mitochondrial localization of the majority of monomeric/misfolded SOD1 was confirmed by double staining with a Tom20 antibody. Extracellular misfolded SOD1 was also observed by exclusion of double staining with markers of CNS cell types (anti-GFAP, anti-Mac2).

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Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 36–38

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SESSION 7B CARER SUPPORT

C46 THE IMPACT OF MOTOR NEURON DISEASE ON CARERS AND THEIR EXPERIENCE OF SERVICE PROVISION: A SYSTEMATIC REVIEW AND DEVELOPMENT OF A DISEASE-SPECIFIC QUESTIONNAIRE

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Background: Carers of people with motor neuron disease may find their dedication affects their health and lifestyle, which may prevail beyond the patients' death. Health and social services, as well as voluntary organizations, are able to assist and support the carer in various ways. Research can help identify the needs of carers thereby improving service provision and possibly the experience of the carer.

Objectives: To develop a questionnaire which will 1) examine the impact of caring on the health of carers, 2) examine the relationship between self reported patients' health (ALSAQ-40) and that of the carers, 3) examine the extent that service provision can reduce the demands upon carers and consequently benefit their health status.

Methods:

Review. Both quantitative and qualitative research was collected for the 10-year period from 1994 to 2004. Searches were made from online databases, grey literature and personal communications to authors. Thirty-two key texts were retrieved in total.

Qualitative study. Recruitment took place via a specialist MND clinic in the UK. Thirty-seven carers were approached by mail and 23 agreed to participate. Twenty-two were interviewed individually and 11 attended focus groups. All discussions were transcribed verbatim and analysed using a qualitative software package.

Questionnaire. Questions were devised from the themes which emerged from the analysis.

Results:

Systematic review. For the period 1994–2004 nearly two-thirds of studies did not use standardized questionnaires and much of the evidence collected is descriptive and diverse. Due to the nature of the disease, many sample sizes are small (58% have sample sizes $\leqslant 50$) and this makes generalization difficult. A disease-specific questionnaire may improve the overall quality of research and allow for comparison between studies.

Qualitative study. Themes were identified concerning employment; assisted daily living and use of equipment; finances and expenses; mental and physical health; social support, social activities and quality of relationship with patient. Issues with service provision include diagnosis; value of information given; voluntary organizations and support groups; health professionals and respite care. There are also issues about being identified as a carer and looking after themselves while caring for someone else.

Questionnaire development. A questionnaire has been developed and in response to a draft and a pilot questionnaire survey, alterations regarding layout and wording of some questions have been made. A long form questionnaire has been developed and a large scale postal survey is being undertaken to determine the questionnaire's measurement properties and to refine the instrument and scoring algorithms.

Discussion and conclusions: The systematic review has shown a paucity of written documentation on the experience of looking after someone with MND. Data collected are diverse and sometimes contradictory. Comparison across studies is difficult due to the diversity of instruments and styles used. Sample sizes are often small and generalization is difficult. The review confirmed a need for a disease-specific questionnaire for carers. A disease-specific questionnaire is in the process of being developed which will further the understanding of the carers' experience of looking after someone with MND.

C47 THE IMPACT OF ENTERAL NUTRITIONAL SUPPORT ON THE QUALITY OF LIFE OF ALS PATIENTS AND THEIR PRIMARY CAREGIVERS

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Background: The implementation of enteral feeding is a standard of care in the treatment of ALS, yet this support is often left until late in the course of the illness. Perhaps because of this, the impact of enteral nutrition on survivorship remains controversial, and the benefit of enteral nutritional support may not necessarily be found in an enhanced survival but rather in an enhancement of quality of life.

Objectives: The purpose of this study is to determine if the implementation of enteral nutritional support affects 1) the quality of life of ALS patients, and 2) the caregiver burden and/or the quality of life of caregivers.

Methods: A multicentre, prospective, cohort study was conducted. A total of 64 patients with clinically definite ALS (24 of whom subsequently had a feeding

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045177

tube inserted) completed a general quality of life measure (SF-36), a generic health status measure (Euro QoL EQ-5D), a mental health instrument (Hospital Anxiety and Depression Scale), a mastery instrument (Locus of control), and a disease specific physical functioning instrument (ALSFR-S) at the first clinical visit. About six months later, at the second visit, a total of 50 patients (20 had a feeding tube in situ) completed these instruments. Data in the aforementioned instruments were available for 57 and 46 caregivers at the first visit and the second visit, respectively. In addition, the caregivers also completed the Zarit Burden instrument.

Results: At the fist visit, patients who subsequently had a feeding tube inserted had significantly lower Mental Health scores (SF-36) (difference −12.08, 95% CI −21.09 to −3.06). At the second visit this difference disappeared, at which time the patients with a feeding tube had higher anxiety scores than those without tubes (difference −1.63, 95% CI −3.22 to −0.05). Also, at the second visit, the patients with a feeding tube had lower locus of control scores (suggesting a more internal locus of control), with the difference between the two groups of 3.85 (95% CI 0.56–7.14). There was no significant difference between the two groups in terms of other quality of life scores. There were no group differences for the caregivers at the first visit. However, at the second visit, the caregivers of patients with a feeding tube had significantly higher EQ-5D scores (difference 0.094, 95% CI 0.001–0.187) and higher depression scores (difference 0.968, 95% CI 0.062–1.874) than caregivers of patients without feeding tubes.

Discussion: Insertion of a feeding tube in patients with ALS is associated with an internal locus of control in the patient (the belief that one can control what happens to him/her) and an increased quality of life in the caregivers. However, there is increased patient anxiety and depression in the caregiver.

Acknowledgement
This research was supported by ALSA

C48 CAREGIVER TIME USE: A PILOT STUDY IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: The impact of ALS is not limited to the patients: in fact, as the disease becomes more severe, the patients become increasingly dependent on caregivers. The ALS caregivers' burden has been demonstrated to be related to patients' clinical disability and on caregivers' psychological factors, such as depression. Recently (1) we have found that the most impaired domain of the Caregiver Burden Inventory (CBI) was the Time-Dependence burden, indicating that ALS caregivers are mostly affected by the amount of time they have to spend in caring. Surprisingly, no studies have been performed devoted to the analysis of caregiver time use in ALS.

Objective: To evaluate the amount of caregivers' time needed for caring for the patients, relating it to patients' disability.

Methods: The amount of time spent for caregiving was evaluated with the Caregiver Activity Time Survey (CATS) (2), an instrument that analytically assesses the time of caring in nine domains (feeding, toileting, bathing, dressing, administering medications, supervision, housekeeping, transportation, other tasks). The caregivers are asked to indicate the total time (hours and minutes) spent for caregiving in a typical day. Patients' disability was evaluated with the ALS-FRS scale. Statistical analysis was performed with multiple regression analysis.

Results: A total of 70 ALS patients were included in the study (28 females, 42 males; mean age, 61.3 years (SD 10.6); mean disease duration 48.8 months (SD 38.3)). The mean number of caregivers for patient was 1.99 (SD 1.3; range 1–8)). There were 53 spouses, 50 children, 16 paid caregivers, six parents, and seven other relatives. The mean time spent for caregiving was 570 minutes per day (SD 512; range 15–3051). The activities which needed more time were housekeeping (156 minutes per day), toileting (83 minutes), and feeding (74 minutes). The total time spent for caregiving was significantly related to patients' disability (r=0.48, p=0.0001). Interestingly, the highest correlation was found with lower limbs disability (r=0.47), the lowest with bulbar disability (r=0.24). Regarding the CATS domains, only toileting, bathing and dressing were significantly related to the total time paid in caregiving. No relationship was found between patients' age or disease duration and caregiving time.

Conclusions: This is the first study which has analysed the caregiver time use in a large population of ALS patients. The amount of time spent in caregiving was related to patients' disability. In particular, it was mostly related to lower limbs disability, indicating that the loss of independence due to the inability to walk and stand has a major impact on the needs of care. This study suggests the importance of the caregiver component of the disease, as assessed by the CATS, as a relevant, albeit indirect, measure of outcome for potential therapies for ALS.

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C49 ENGAGING SOCIAL SUPPORT SYSTEMS TO IMPROVE QUALITY OF LIFE FOR PATIENTS AND CARERS

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Background: The availability of a strong supportive care network for people living with life limiting illness has been positively correlated to reduced stress and burden (1–3).

Objectives: This study examined the availability and importance of support networks to family carers of people living with ALS/MND.

Methods: Seventy-five family carers participated in the carer network scale mail-out survey, and 14 primary carers were interviewed face-to-face, three times over a 10-month period. One-way analysis of variance was undertaken across all items of survey data. Interview data were analysed using NVivo software with the first two levels of grounded theory being applied to frame the analysis.

Results: Statistical significance was apparent across all survey items, however the length of time caring was the main predictor of loss of support and sense of burden. Reductions in social support include items such as: 'caring restricts my social life' ($p<0.038$); 'caring causes difficulties in relationships with friends' ($p<0.013$); 'relatives don't keep in touch as often as I'd like' ($p<0.006$). Coded interview data supported these findings and provided further detail about the circumstances and situations that precipitated the loss of social support.

Conclusions: These results suggest that caregiving requirements impact on people's ability to preserve the relationships necessary to maintain their quality of life and the standard of care they can give to the person living with ALS/MND. Assessment of the psychological and social supportive care needs, as well as the physical needs of carers, is therefore integral to effective care management.

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C50 ATYPICAL FEATURES IN PATIENTS WITH ALS AND THEIR IMPACT ON CAREGIVER BURDEN: FINDINGS FROM THE ALS PATIENT CARE DATABASE

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Background: Some patients with amyotrophic lateral sclerosis (ALS) develop atypical features, which indicate extra-motor abnormalities. The frequency in a large population of patients with ALS is unknown, as is whether their occurrence influences ease of diagnosis, disease course, and caregiver burden. The ALS Patient Care Database collects information on ALS from physicians, patients, and their caregivers, including atypical features and caregiver burden.

Objectives: To determine the frequency of atypical features in ALS patients enrolled in the ALS Patient Care Database between 1995 and 2004, and examine their influence on ALS diagnosis and caregiver burden.

Methods: Information on 4398 patients with sporadic (SALS) or familial (FALS) ALS was analyzed to characterize the: 1) frequency of atypical features; 2) delay to diagnosis; and 3) caregiver burden, based on the Caregiver Burden Score, which is derived from self-reports of caregivers to 17 questions that quantify the burden of caring for the patient.

Results: ALS was sporadic in 4114 (94%) and familial in 284 (6%) patients. One or more atypical features were reported in 658 (17%) patients with SALS and in 53 (20%) patients with FALS. Comparison of SALS and FALS patients, respectively, revealed one atypical feature in (13%, 13%), two atypical features in (3%, 5%), and three or more atypical features in (1%, 1%). No significant difference was seen in the frequency of atypical features between SALS and FALS groups: sensory dysfunction (6%, 8%), cognitive change (4%, 5%), bladder dysfunction (2%, 3%), ataxia (2%, 2%), extrapyramidal features (1.5%, 1%), autonomic dysfunction (<1%, 0%), and 'other' (8%, 11%). Duration from symptom onset to diagnosis was longer in patients with atypical features ($p=0.01$). Caregiver burden was significantly greater if patients had atypical features of cognitive change ($p=0.0003$) and bladder dysfunction ($p=0.003$) and did not reach significance if extrapyramidal ($p=0.05$) or other features ($p>0.05$) occurred.

Conclusion: About 20% of patients with ALS have atypical features, most commonly sensory dysfunction and cognitive change. Although not reaching significance, these tend to be more frequent in FALS patients. Diagnosis of ALS was significantly delayed if patients had atypical features suggesting more uncertainty in making a diagnosis. Burden on the caregiver is greater when atypical features are present, especially with conditions necessitating more intervention and supervision such as cognitive impairment and bladder dysfunction. Attention to these findings by health care professionals and caregivers may improve care for patients with ALS, and patient-caregiver interactions.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 39–41

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SESSION 7C IMAGING AND NEUROPHYSIOLOGY

C51 STATISTICAL MOTOR UNIT NUMBER ESTIMATION AND ALS TRIALS: THE EFFECT OF MOTOR UNIT INSTABILITY

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Background: Motor unit number estimation (MUNE) remains the most attractive physiological surrogate marker to monitor disease progression in ALS. The statistical method of MUNE has a number of potential advantages for use in trials, including a well defined protocol, high test-retest reliability, and clear relationship to survival when employed by experienced neurophysiologists. In two multicenter, double-blind, placebo-controlled trials of ALS, statistical MUNE displayed good reliability and declined linearly with time. However, single motor unit potential (SMUP) amplitude was unchanged over 6 and 12 months, despite modifications made to the method between trials to identify the expected increase in motor unit size over time. We suspected that the reason motor units did not increase over time was amplitude instability of single motor units, based on previous studies showing excess variability in motor units from ALS patients.

Objectives: To determine whether the previously measured increase in motor unit response variability present in patients with ALS is sufficient to confound MUNE using the statistical method.

Methods: Using data from prior studies on motor unit variability, submaximal compound motor action potentials (CMAPs) were modelled using a constant number of motor units of different amplitudes. It was assumed that repeated stimulation resulted in the same motor units being activated to every stimulus. The variance of the CMAPs was calculated, based on the variances of the individual SMUPs. From that variance, the statistical method of MUNE was used to generate an estimate of the SMUP amplitude.

Results: Statistical MUNE calculates SMUP amplitude based on the variability of CMAP amplitude in response to a constant intensity stimulus, assuming that this variability is due completely to a variable number of motor units responding. Our model requires that the same sample of motor units fire to every stimulus, so that the correct estimate of SMUP amplitude is 0. However, the intrinsic SMUP variability present in ALS motor units resulted in SMUP estimates of 48–70 uV, well within the range of normal motor unit sizes.

Discussions and conclusions: In ALS patients, the increased variability of motor units renders the statistical technique of MUNE invalid. It is likely that in prior clinical trials, the failure of SMUP amplitude to increase with time was a function of unstable single motor units being mistakenly identified as multiple motor units firing in a statistical fashion. While motor unit instability poses a problem for all methods of MUNE, the statistical method is particularly vulnerable, as it is the variability of the response to a repeated stimulus that forms the basis for determination of SMUP amplitude.

C52 EVIDENCE FOR CORTICAL DYSFUNCTION IN ALS: RESULTS OF AN MEG STUDY

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Background: The motor areas of the brain have long been known to be involved in ALS but the timing of onset and the magnitude of cortical involvement have only recently been studied. Magnetoencephalography (MEG) is a sensitive technique that can detect changes of cortical electrophysiology and map sources of abnormal slow wave activity.

Objectives: The intent of this study is to determine whether MEG could detect evidence of altered cortical function in patients with ALS. A secondary objective, having documented abnormal cortical function, focuses on the timing and anatomic extent of these abnormalities.

Methods: Non-invasive MEG was obtained in patients with clinically definite ALS and in control subjects of similar age. Whole head 148 channel MEG of spontaneous brain activity is recorded for 15 min in awake state. Abnormal slow wave activity (focal, high amplitude, 1–7 Hz) is identified visually. The slow wave dipole source solutions are generated from a single equivalent current dipole model. Qualifying dipole solutions meeting pre-selected confidence volume and additional statistical stringency measures are counted and then mapped to anatomic brain regions by co-registration with the subject's own or reference brain MRI.

Results: Focal, high amplitude, slow wave activity is present in 6/6 ALS subjects and 0/6 control subjects. The abnormal waveforms are 3–6 Hz with a persistent intermittent pattern. The slow wave source generators localize to the frontal lobes in all ALS subjects, and to varying

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045186

degrees in the temporal lobes. Frontal slow wave source generators span the supplementary motor and premotor areas, the anterior to mid-cingulate bilaterally, operculum, and insula. This multifocal bi-hemispheric abnormal cortical electrophysiology is also present at the presentation of early stage (short duration) disease.

Conclusions: The finding of abnormal slow wave activity in a representative population of ALS patients suggests early and widespread involvement of the cortex. In all patients, frontal involvement is seen and to varying degrees concomitant temporal involvement. These observations need to be extended to a larger group of patients. These findings are consistent with the results obtained with PET scanning using a benzodiazepine ligand. Both MEG and PET scanning could have broad research and clinical application. For example, MEG might be useful for making the diagnosis of ALS when patients exhibit only lower motor neuron findings at the time of presentation.

C53 IMAGING IN PATIENTS WITH PURE AND COMPLICATED HEREDITARY SPASTIC PARAPARESIS: 3D MRI MORPHOMETRY OF THE BRAIN AND UPPER SPINAL CORD PLANIMETRY

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Hereditary Spastic Paraparesis (HSP) encompasses a group of inherited neurodegenerative disorders of heterogeneous phenotype that share the principal clinical feature of progressive weakness and spasticity predominantly affecting the lower limbs. Conventionally, they are categorized into pure forms (pHSP) and complicated forms (cHSP), the latter characterized by the presence of additional major clinical features.

In the present study, 33 HSP patients suffering from pHSP (n=22) or cHSP (n=11) were investigated using different magnetic resonance imaging (MRI) techniques. MRI data of the brain, including a volume-rendering 3-D data set, and of the upper spinal cord were acquired. Besides standard visual assessment, the diameters of the cervical and thoracic myelon were measured by planimetric techniques. Cerebral 3D MRI was analyzed by use of the observer-independent whole-brain based technique of voxel-based morphometry (VBM), both in comparison with an age-matched normal database and with respect to a comparison between pHSP and cHSP. Moreover, relative global brain volumes were analyzed by the automated brain parenchymal fraction (BPF) method.

Despite the heterogeneity of the cHSP phenotypes, the standardized BPF analysis showed characteristic patterns of the global cerebral alterations, both with respect to BPF itself and to the grey and white matter sub-fractions. VBM demonstrated regional grey and white matter volume decreases (atrophy) in both HSP subtypes with the most robust alterations located in the corpus callosum. The upper spinal cord was significantly decreased in diameter with respect to controls. Our results demonstrate the involvement of extrapyramidal cerebral and spinal CNS structures in a sizeable group of pHSP/cHSP patients and give insight into pathophysiological basics and, thus, might help to improve our understanding of the characteristics of this heterogeneous disorder.

C54 DEVELOPMENT OF UPPER MOTOR NEURON INVOLVEMENT IN ALS: A LONGITUDINAL MRI STUDY USING DIFFUSION TENSOR IMAGING AND VOXEL-BASED MORPHOMETRY

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Background: Extent and distribution of upper motor neuron (UMN) involvement in ALS has been described in post-mortem brains. Little is known about the extent and development of UMN degeneration *in vivo*, and it is unclear whether cortical atrophy is present at all early in the course of ALS and how it develops over time. Region of interest based analyses have suggested neuronal loss in central regions, and signal abnormalities were found in the corticospinal tract (CST) by computerised analyses in diffusion tensor imaging (DTI). Detection of cortical atrophy in the sensorimotor and frontal cortex as well as reduction of the anisotropy in the CST has been demonstrated recently by our group using voxel-based morphometry (VBM) and DTI analysed on a voxel-by-voxel basis.

Objective: The aim of the present MRI study was to describe the development of UMN involvement in ALS patients. We used voxel-based morphometry and Diffusion Tensor Imaging to detect region-specific changes in grey matter volumes (VBM) and differences in fractional anisotropy (FA) of white matter tracts (DTI).

Methods: High-resolution anatomical MRI and DTI (15 directions) were performed in 21 patients with ALS at baseline and in 13 six months later, and 23 age-matched controls on a 1.5 T GE-scanner. Images were analysed on a voxel-by voxel basis using SPM2. Group comparisons of regional grey matter and FA and were made using ANCOVA with the global mean voxel value as confounding factor. Longitudinal assessments were carried out using paired t-tests.

Results: Compared with controls, ALS patients at baseline showed a relative decrease in GMV in the pre- and post-central gyrus, inferior parietal lobe and the middle frontal gyrus. In addition, there was reduced FA in the CST bilaterally. At follow-up after six months, the relative decrease in GMV had progressed to cingulate areas and the cerebellum. In DTI, FA had decreased in the genu of the internal capsule and CST descending into the brainstem bilaterally.

Conclusion: The present study confirms our previous findings of largely central atrophy extending to frontal

areas and FA reductions in the CST in a second cohort of patients. On follow-up examination, the atrophy had spread to larger areas of the sensorimotor cortex and included cingulate and cerebellar structures which is in line with recent histopathological findings. In addition, CST abnormalities extended into the brainstem. Our results consistently show grey matter atrophy in sensorimotor, parietal and frontal regions which increases during the course of ALS. In groups of patients, voxel-based morphometry and DTI may be used as a monitoring tool in future clinical trials.

C55 QUANTITATIVE EVALUATION OF FLAIR-MR IMAGES CORRELATES TO SPASTICITY SCORE BUT NOT TO CORTICAL EXCITABILITY

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Background: Degeneration of the upper motor neuron is often clinically masked by the degeneration of the lower motor neuron in ALS. MR imaging of the brain is commonly used to exclude diseases mimicking ALS. However, quantification of the subcortical precentral gyrus FLAIR-signal revealed a significant hyperintensity in ALS patients which increased in the follow-up examination (1,2).

Objectives: The aim of our study was to compare quantified FLAIR-MR images with a standardized clinical examination of upper motor neuron and cortical excitability.

Methods: In 30 ALS patients (16 males, 14 females, 59.6 ± 9.6 years, probable or definite ALS) we performed axial FLAIR-MR images and calculated the 'contrast to noise ratio' (CNR) of regions of interest within both subcortical precentral gyri (1). In addition, all patients where clinically investigated using the 'spasticity score'. Cortical inhibition and facilitation was determined using paired transcranial stimulation.

Results: The CNR of the right and left subcortical precentral gyrus correlated significantly to the contralateral spasticity score (CNR right – spasticity left, $r=0.437$, $p=0.016$; CNR left – spasticity right, $r=0.488$, $p=0.006$; CNR mean – spasticity bilateral, $r=0.613$, $p=0.005$). We found no correlation of the subcortical precentral CNR to cortical inhibition ($r=-0.216$, $p=0.375$). Short interval cortical inhibition showed only a trend to the spasticity score ($r=0.421$, $p=0.057$, $n=21$).

Discussion and conclusion: Cortical excitability changes seem to occur independently of morphological changes demonstrated by FLAIR-MR images. This finding might be due to the complex changes of cortical excitability in ALS. For clinical purposes however, the high correlation of the subcortical precentral CNR with the spasticity score demonstrates a possible clinical value. CNR measurement might be useful as an objective marker of upper motor neuron changes in clinical trials.

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C56 IMPAIRED CARDIOVAGAL AND INCREASED VASOMOTOR RESPONSES IN EARLY-STAGE AMYOTROPHIC LATERAL SCLEROSIS

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Background & objectives: Previous studies have indicated autonomic nervous system (ANS) involvement in amyotrophic lateral sclerosis (ALS).

Subjects and methods: ECG and continuous tonometric blood pressure were monitored in 25 ALS patients (age 56.2 ± 14.5 years, time from onset 11.2 months) and 42 age-matched normal controls (53.16 ± 13.14 years) during paced breathing (six breaths per min), Valsalva manoeuvre, and upon active standing. Expiratory/Inspiratory (E/I), Valsalva and 30:15 ratios were calculated. Sensitivity of the baroreflex was assessed on the basis of Valsalva manoeuvre by calculating the following latencies: tachycardia (TL), bradycardia (BL), blood pressure recovery (BpL) and overshoot (OvL) latencies (1).

Results: No differences in resting heart rate and BP were found between groups. The E/I ratio was significantly lower in the patients (1.19 ± 0.12 vs. 1.38 ± 0.15, $p=0.0003$) but Valsalva and 30:15 ratios were similar in both groups. Valsalva latencies TL (2.50 ± 0.53 vs. 3.50 ± 1.3, $p=0.002$) and BpL (2.50 ± 0.53 vs. 2.83 ± 1.11, $p=0.02$) were significantly shorter in patients, as was the duration of early phase II (5.50 ± 1.7 vs. 7.58 ± 1.32, $p=0.03$). BL and OvL were not different between groups. By the end of the phase II of the Valsalva manoeuvre, systolic BP was significantly higher in patients than in controls (142.4 ± 25.9 vs. 126.5 ± 17.9, $p=0.001$).

Conclusions: These results demonstrate mild parasympathetic impairment and hyperactive peripheral sympathetic activity in patients with ALS. These findings suggest a combined central and peripheral involvement of the ANS and could be useful in the differentiation of ALS from other clinically similar disorders.

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Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 42–45

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SESSION 8A THERAPEUTIC STRATEGIES

C57 THE EFFECTS OF ANGIOGENIN ON MOTOR NEURON DEGENERATION

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Background: Investigation of genes coding for hypoxia-inducible factors in characterized Irish, Scottish and American populations of sporadic ALS patients has led to the identification of a series of novel disease-specific mutations in angiogenin, a hypoxia-responsive peptide not hitherto associated with ALS pathogenesis (1). Recent studies have shown that other hypoxia-inducible factors, including VEGF and IGF-1, are neuroprotecitve to motor neurons (2,3).

Objective: We studied the effect of hypoxia on the expression of angiogenin in cultured motor neurons, and examined the neuroprotective properties of exogenous angiogenin *in vitro*.

Methods: Using quantitative PCR, Western blotting and immunohistochemistry, we studied the expression of angiogenin in motor neurons in response to hypoxic conditions of 1%, 3% and 5% O_2. The neuroprotecitve effect of angiogenin treatment was tested using *in vitro* models of motor neuron degeneration, where primary motor neuron cultures were exposed to either hypoxic or excitotoxic conditions. Motor neuron survival in each paradigm was determined using LDH and MTT assays as well as by trypan blue exclusion.

Results: Angiogenin expression was more abundant in the CNS, and a differential expression of angiogenin was shown between the nervous system and other organs. Under hypoxic conditions motor neurons and glia significantly increased angiogenin expression. In motor neuron cultures exposed to either hypoxic conditions (1% O_2 for 24 hr) or excitotoxic conditions (AMPA 50uM for 24 h), co-treatment with angiogenin was found to significantly increase motor neuron survival. Following exposure to hypoxic conditions motor neuron survival is reduced to 60% ($\pm$3.2 S.E.M, n=3), however co-treatment with angiogenin significantly increased motor neuron survival to 82% ($\pm$4.9 S.E.M, n=3).

Conclusion: These studies demonstrate that angiogenin is expressed preferentially in the nervous system where it has an important role as a hypoxia inducible factor. Angiogenin also is neuroprotective to motor neurons exposed to either hypoxic or excitotoxic conditions.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045195

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C58 TREATMENT WITH MECHANO-GROWTH FACTOR (MGF) RESCUES MOTOR NEURONS AND DELAYS DISEASE PROGRESSION IN SOD-1^{G93A} MICE

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Background and objectives: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by motor neuron degeneration and muscle paralysis. Despite considerable research, there remains no effective treatment for this disease. However, recent evidence has highlighted the therapeutic potential of neurotrophic factors in ALS. In particular, Insulin-Growth-Factor 1 (IGF-1) has been shown to increase lifespan and delay disease progression in the SOD-1^{G93A} mouse model of ALS. In this study we examined the potential therapeutic benefits of Mechano-Growth Factor (MGF) in the SOD-1^{G93A} mouse model of ALS. MGF is produced by alternative splicing of the *IGF-1* gene and is known to have both myotrophic and neurotrophic effects, and may have a greater efficacy than IGF-1.

Methods: The hindlimb muscles of SOD-1^{G93A} mice were injected with a mammalian expression plasmid containing the MGF cDNA sequence at 70 days of age, an early symptomatic stage of the disease. At 120 days, hind limb muscle force and motor unit survival were assessed by *in vivo* physiological recordings. The muscles and spinal cord were subsequently removed and processed to assess motor neuron survival and muscle histology.

Results: At 120 days of age, the hind limb muscles of untreated SOD-1^{G93A} mice were significantly weaker than in normal littermates and the maximum force of the tibialis anterior (TA) muscles was only 13.5 g ($\pm$2.5, n=8) compared to 126g ($\pm$16.58, n=7) in wild-type mice. However, in MGF treated SOD-1^{G93A} mice, the muscles were significantly stronger and the force of TA was 36.6 g ($\pm$5.58, n=6, $p \leqslant 0.021$). Moreover, significantly more motor units survived in MGF treated SOD-1^{G93A} mice than in their untreated SOD-1^{G93A} littermates. Extensor digitorium longus (EDL) muscles are normally innervated by 30 ($\pm$2.43, n=7) motor units. In untreated SOD-1^{G93A} mice only 10 ($\pm$0.75, n=8) motor units survived at 120 days. However, in SOD-1^{G93A} mice treated with MGF,

EDL had 15 ($\pm$1.89, n=7) motor units. This improvement in motor unit survival was reflected in an increase in motor neuron survival in the spinal cord of MGF treated SOD-1^{G93A} mice in which 355 ($\pm$16.65, n=4) motor neurons survived in the segment of the sciatic motor pool examined, compared to only 199 ($\pm$15.13, n=4) in their untreated SOD-1^{G93A} littermates (p=0.029).

Conclusions: These results show that treatment with a mammalian expression plasmid containing MGF cDNA, rescues motor neurons and improves muscle function in a mouse model of ALS. MGF may therefore be of therapeutic value to patients with ALS.

Acknowledgement
We are grateful to the Motor Neurone Disease Association, BBSRC and Brain Research Trust for support.

C59 SYNERGY OF IGF-1 AND EXERCISE IN ALS AND INVOLVEMENT OF MUSCLE IN DISEASE PATHOGENESIS

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the neuromuscular system resulting in paralysis and ultimately death. Currently, no effective therapy is prescribed for patients; however, several therapeutic strategies are showing promise. Either exercise or treatment with a gene therapy; adeno-associated virus/insulin-like growth factor-1 (AAV-IGF-1) alone has therapeutic benefits in an ALS transgenic mouse model.

Objectives: To test the effects of exercise, in the form of running wheel, on ALS progression and to determine the extent of synergy between exercise and ALS. Furthermore, we tested the involvement of muscles in disease progression.

Methods: Animals were placed in an activity monitoring system beginning at 40 or 90 days of age, and a computer-controlled wheel revolution monitor assessed individual or group running. At 90 days of age, randomly assigned male and female transgenic mice with the G93A human SOD1 mutation were injected bilaterally with 15 ul of AAV-GFP or AAV-IGF-1 into the intercostal muscles and quadriceps muscles using a Hamilton syringe. Mice were observed daily for survival. Testing of motor function using a rotarod device began at 40 days of age. To determine mortality in a reliable and humane fashion, we used an artificial endpoint, defined by the inability of mice to right themselves 30 sec after being placed on their sides.

Results: We have shown that activity duration affects the therapeutic benefit associated with exercise, with 6- and 12-h exposure to a running wheel providing significant motor function benefits and increased survival. Remarkably, a combination of IGF-1 gene delivery and exercise has profound effects on survival and function nearly doubling the animal's lifespan, indicative of synergistic effects with exercise and IGF-1. Furthermore, we show that the combination of exercise and IGF-1 is working on different downstream mediators within the apoptotic cascade. We demonstrate gene expression changes in signaling molecules within the apoptotic cascade, such as Bcl-xL, Bcl-2, PI-3 kinase and AKT activation.

Discussion: Our results indicate that a drug treatment in combination with appropriate exercise may provide the most promising therapy for ALS to date. Further work is defining the contribution of the muscle and effects of IGF-1 and exercise on muscle pathology in ALS.

C60 HUMAN BONE MARROW MESENCHYMAL CELL-DERIVED ASTROCYTES: A NEUROPROTECTIVE APPROACH TO ALS

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Background: Recently it has been proved that non-neuronal cells (astrocytes) may exert a neuroprotective effect on neighbouring SOD1 motor neurons (MNs) thus increasing lifespan survival in the ALS mouse model (1). Astrocyte replacing/transplantation for the production of trophic support for dying MNs within the spinal cord would be an appealing and realistic approach towards an ALS clinical therapy. Therefore, we decided to test mesenchymal stem cells (MSCs) (easily derivable from patient bone marrow) transdifferentiation potential towards the neuro-glial cell phenotype.

Objectives: The purpose of the present study was to verify human adult MSCs effective differentiation towards the neuro-glial phenotype *in vitro* and *in vivo*, with the exclusion of alternative explanations (i.e. cell fusion).

Methods: Thirty-two different MSC samples were investigated after several induction protocols *in vitro*. Extensive characterization was performed on human cells by combining morphological observations and gene expression both at transcription (RT-PCR) and translational level (immunocytochemistry). Transdifferentiation potential was further confirmed *in vivo* by transplantation in the cerebral cortex of post-natal nude mice.

Results: After several treatments, such as co-culturing, addition of stem cell- or astroblast-conditioned media, MSCs show alteration of their cellular morphology. Particularly, we detected astroglial-like morphology in addition to GFAP immunocytochemical labelling and activation of specific neuronal mRNAs (such as MAP2 and NFM) after co-culturing with astroblasts, both in the absence or presence of cell contact. Grafted human MSCs were equally demonstrated to express GFAP and neurotrophin receptors while human neurotrophin release was detectable even at 45 days post-transplantation.

Conclusions: In summary, our data show that MSCs possess a neuroglial potential since they can modify their morphological features. Notably we detect astroglial specific protein expression after treatment *in vitro* and after transplantation *in vivo* with neurotrophin production. These data, combined with the recent hypothesis on the essential role played by astrocytes in pathological processes, may open new prospect for cell therapy in ALS, although further investigations and pre-clinical data on TgSOD1G93A mice or other animal models are needed to better characterize neuroprotection as a reparative mechanism for the treatment of ALS patients.

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C61 A HIGH CALORIFIC KETOGENIC DIET AS A POTENTIAL NOVEL THERAPEUTIC INTERVENTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: There is evidence that ALS patients have a chronically deficient intake of energy and that augmentation of energy intake may be beneficial to disease progression.

Objective: We explored the role of high calorific high-fat, low-carb ketogenic dietary regimen in the G93A human superoxide dismutase (SOD1) mouse model of ALS. Previous evidence indicated that similar ketogenic dietary regimen can neuroprotect against glutamate neurotoxicity *in vivo* (1) and beneficially influences experimental neurodegeneration (2).

Methods: The macronutrient calorific composition of the high calorific ketogenic diet was 60% fat (from lard), 20% carbohydrate (refined sucrose) and 20% protein; in parallel a control G93A mouse group was fed a normal diet consistent with a standard rodent laboratory diet. Both diets contained a standard vitamin and mineral mix with all essential nutrients, and an equal cholesterol content within the range of cholesterol reported for most mice vivaria diets.

Results: We found that a high calorific dietary regimen, achieved by exposing G93A mice to a high-fat, low-carb ketogenic diet starting at 50 days of age in male G93A ALS mice, had an overall significant slower decline in ALS type motor impairment compared to G93A mice fed normal diet as assessed by Rotarod assay (ANOVA, $F=16.7$, $p<0.0001$). Moreover, the slower decline in motor impairment in G93A mice fed high calorific diet coincided with a slower rate of weight loss relative to G93A mice fed a normal diet. Finally, we found that survival was significantly increased in the G93A mice fed a high calorific diet relative to G93A ALS mice fed normal diet (ANOVA $F=11.8$, $p<0.001$). Most importantly, the ketogenic feature of the high-calorific diet was confirmed in G93A mice by showing >2.5-fold elevation in the circulating ketonic body D-β-3 hydroxybutyric acid ($p<0.05$). This correlated with neuroprotection of lumbar (L3–L5) ventral horn motor neurons, relative to G93A SOD-1 mice fed a standard rodent laboratory diet. Consistent with these results in ongoing studies in the laboratory we found that treatment of NSC-34 motor neuron-like cells with D-β-3 hydroxybutyrate (7 mM, four days pretreatment) significantly protected against mutant SOD-1 mediated neuronal death assessed by LDH release assay, 24 h after viral infection with a mutant SOD-1 construct.

Conclusion: The study provides experimental evidence that prophylactic augmentation of calorific intake can significantly delay motor dysfunction in the SOD1-G93A transgenic mouse model of ALS.

Acknowledgement
This study was supported by MDA and Department of VA ALS research grant to GMP.

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C62 MODELING A THERAPY FOR FAMILIAL ALS

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Background: A potent antisense therapeutic (Isis oligo 333611) that targets the expression of both mutant and wild-type SOD has been identified from a panel of 80 oligonucleotides. This may be a putative treatment for the SOD variant of FALS, which is an example of a neuroproteinopathy that results from an unknown toxic property of the mutant protein. In a murine model of

FALS disease, it has been shown to correlate with the amount of mutant protein. In the G93A rat model we have recently demonstrated increased survival after administering 333611 chronically via the intraventricular route. Intraventricular (ICV) or intrathecal (IT) administration of optimized antisense oligonucleotide (ASO) drugs have been shown to significantly reduce target protein levels in rats.

Objective: To determine the optimal route of administration and dosing regimen for ASOs in non-human primates to support clinical trials in FALS patients.

Methods: Prior to catheter implantation, rhesus monkeys were intubated and administered isoflurane by inhalation. In four animals, cannulas were placed in the left lateral ventricle and in two animals a cannula was placed in the lumbar subarachnoid space. Five days after recovery 1 mg of ASO or saline was infused continuously via an external pump to animals via the ICV route and the remaining two animals received ASO via the IT route. Blood and spinal fluid were collected at the beginning and end of treatment and brain and spinal cord were rapidly removed for analysis following sacrifice. ASO concentration was determined in representative neural tissues by capillary gel electrophoresis and tissues were fixed for histologic evaluation.

Results: ASO tissue concentrations ranged from a low of 17 ug/g in the left hippocampus to a high of 118 ug/g in the right frontal/parietal cortex in animals treated intracranially. Spinal cord levels ranged from a low of 35 ug/g in the lumbar cord of one animal to a high of 95 ug/g in the thoracic cord of another. In contrast lumbar tissue levels were above 180 ug/g in one animal receiving IT treatment whereas brain levels generally ranged from 20 to 30 ug/g of tissue. In addition, ASO was detected in both liver and kidney. Cellular uptake of ASO was detected by immunohistochemistry in the spinal cord by both routes of administration but, as indicated by CGE analysis, apparent cellular uptake was greater after IT administration.

Discussion: As is the case in rodents, ASOs are widely distributed in the CNS after IT or ICV administration in non-human primates. ASO concentrations achieved in non-human primates by the ICV or IT route are in the range that exhibit pharmacological activity in rodents. As expected, the route of administration does influence the relative distribution of ASO in the CNS, with spinal levels being highest after IT administration and intracranial levels being higher following ICV administration. The PK results in subhuman primate should provide guidance regarding the dosing requirements in a human clinical trial.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 46–48

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SESSION 8B COGNITION

C63 ALS AND FRONTOTEMPORAL LOBAR DEGENERATION (FTLD): DYSFUNCTIONAL FAMILY OR DISTANT RELATIVES?

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Once considered to be a discrete neurodegenerative disorder restricted to the motor system, ALS is increasingly recognized to be a syndrome in which multiple neuronal systems can be involved. Paramount among this has been the recognition that ALS may be associated with a frontotemporal lobar degeneration. The clinical spectrum of such dysfunction can include behavioural impairments (ALS with behavioural impairment: ALSbi), cognitive dysfunction in which impairments in verbal fluency and list generation may be the earliest evidence of dysfunction (ALS with cognitive impairment: ALSci), a more florid frontotemporal dementia meeting the Neary criteria (ALS-FTD) or a more widespread dementia (ALS with dementia: ALS-D). It remains unknown whether these are discrete clinical presentations, or part of a continuum or spectrum. Depending on the nature of the neuropsychological studies, the incidence among the ALS population of such deficits may range from 30% to 50% or more, with the vast majority exhibiting only subtle deficits.

The neuropathological and molecular basis of this process is consistent with a frontotemporal lobar degeneration (FTLD), leading to the concept that there is significant overlap between the FTLDs and ALS on a biological basis. The neuropathological hallmark includes superficial linear spongiosus, both a microglial and astrocytic proliferative response within the frontotemporal neocortex, and the presence of ubiquitinated intraneuronal aggregates within the dentate fascia. In common with many of the FTLDs, FTLD in ALS is a tauopathy as evidenced by the presence of intraneuronal and glial tau aggregates, tau immunoreactive neuritic processes and argyrophilic grains throughout the affected regions. Although these features are shared with many of the tau-based FTLDs, the molecular signature of FTLD in ALS is unique with normal expression levels of 3R and 4R transcripts, increased 3R and 4R insoluble tau protein, and hyper-phosphorylation of tau protein with unique phosphoepitopes.

The clinical and biological implications of FTLD in ALS are considerable. Clinically, patients with FTLD and ALS are less likely to participate in interventional therapies such as non-invasive ventilation and are thus in a poorer prognostic subgroup. Biologically, this observation dispels the notion of ALS as a single disease entity and confirms the clinical hypothesis that ALS is syndromic with the potential for biological overlap between ALS and the FTLDs. Within the rubric of the FTLDs, a distinctive molecular signature for ALSci exists. The recognition of FTLD concurrently with ALS will have design and interpretive implications for therapeutic trials in ALS.

Acknowledgement: This work was supported by the ALS Society of Canada, the ALS Association (ALSA) and the McFeat family fund.

C64 AN FMRI INVESTIGATION OF THE NEURAL BASIS OF EMOTIONAL LABILITY IN MOTOR NEURON DISEASE (MND)

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Background: Emotional lability (EL) is seen in ~20% of cases of MND although its neural basis has not previously been studied. The association of EL with pseudobulbar symptoms in MND had led to the assumption that loss of control of laughter/crying resulted from degeneration of corticobulbar pathways disrupting the execution of motor programmes for emotional expression. However, recent evidence for extra-motor prefrontal cortex (PFC) involvement in a proportion of non-demented MND patients, affecting associated inhibitory executive functions, combined with findings that such cognitive dysfunction may be more common in patients with bulbar symptoms, raises the possibility that, in MND, EL represents the loss of inhibition of emotional expression as a result of degeneration of PFC regions mediating emotional control.

Objectives: To explore the neural basis of the control of emotional expression, and to attempt to distinguish between these alternative aetiological hypotheses for EL in MND.

Methods: A functional magnetic resonance imaging (fMRI) paradigm was designed to evaluate the inhibition of emotional expression in response to amusing compared to neutral film stimuli. After careful testing in off-line trials and an fMRI pilot, it was then administered to emotionally labile MND patients (MND-EL, $n=7$), non-labile MND patients (MND-NEL, $n=7$), and healthy controls ($n=8$) during fMRI scanning.

Results: Significant increases in brain activation were recorded for MND-EL patients compared to the other two groups during the emotion inhibition task. The largest and most significant cluster was observed in the left medial frontal gyrus (Brodmann Area (BA) 9/10), with further smaller areas of increased activation affecting the superior

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045203

and middle temporal gyri (BA 21/22), predominantly on the left.

Discussion and conclusions: Our findings suggest that EL in MND is not associated with PFC dysfunction, but instead implicates recruitment of left hemisphere medial PFC regions in the control of emotional expression. While the excess activation in BA 9/10 seen in the MND-EL group may represent attempts to suppress a labile reaction caused by dysfunction lower down in the neural circuit mediating emotional expression, ongoing studies may clarify whether the identification of regions of cortical cell loss can further the understanding of cerebral abnormalities underlying EL in MND.

C65 MAGNETIC RESONANCE (MR) DIFFUSION TENSOR IMAGING (DTI) AND SPECTROSCOPIC IMAGING (MRSI) DETECT WIDESPREAD FRONTAL LOBE INVOLVEMENT IN ALS

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Background: ALS is a disease that affects mainly motor neurons. Various MR imaging methods were used in the past to visualize the disease involvement in motor cortex and corticospinal tract. It has been known from pathology, PET studies, and volumetric analysis that other regions of the brain, particularly the frontal lobe, are affected in ALS.

Objective: To determine the extent of brain alterations in ALS using MR DTI and MRSI.

Methods: We studied 27 consecutive clinically non-demented ALS patients and 12 age-matched controls. The subjects were imaged with a clinical 1.5 T MR scanner and quadrature head coil. A single-shot EPI diffusion tensor sequence is used to collect imaging data from 30 slices covering the entire brain. The slice thickness was 5 mm, FOV 22 cm; the imaging matrix was 128×128. We collected diffusion-weighted images from 26 gradient directions, and six images without diffusion weighting. A total of 32 images per slice were acquired. Using all the images, we calculated the components of the diffusion tensor for every pixel. Maps of average diffusion constant (Dav) and diffusion anisotropy (FA) were then calculated. Using SPM (Statistical Parametric Mapping package) and MATLAB, we warped the diffusion tensor images from each subject into an image template. Using a *t*-test, we then computed the regions that were either increased in Dav or decreased in FA in patients in comparison to controls. We then overlaid these regions onto either axial Dav or FA maps or 3D white matter volume rendered from the subject group. MRSI was also recorded on the same 1.5 T MR scanner with a highly optimized and almost fully automated multislice MRSI pulse sequence, in 27 min using a 240-mm field of view, a TR of 2300 ms, 32×32 circularly applied phase-encoding steps, and one excitation per phase-encoding step. N-acetylaspartate concentration was measured in multiple voxel localized within the anterior frontal cortex and the visual cortex in the second topmost of four slices recorded. Each voxel had a nominal size of 0.83 cc, allowing multiple uncontaminated spectral voxels.

Results: Our methods successfully visualized abnormal MR DTI variables in motor cortex and pyramidal tracts in ALS patients. In addition, we were able to show statistically significant ($p < 0.001$ compared to controls) extensive frontal lobe regions that are involved by the disease process in the group of patients we studied. N-acetylaspartate concentration in the frontal cortex of ALS patients was 6.6 ± 1.6 on the right (controls 8.2 ± 2.0, $p = 0.009$), 6.4 ± 2.1 on the left (controls 8.2 ± 2.1, $p = 0.022$), and the mean of both cortices in ALS was also significantly reduced ($p = 0.007$). There were no differences in N-acetylaspartate concentrations in visual cortex between ALS and controls.

Discussion and conclusion: MR DTI visualized areas in extensive frontal lobe that are involved in ALS in addition to motor cortex and corticospinal tracts. These changes also involve frontal cortical neurons by measuring N-acetylaspartate concentration. To the best of our knowledge, this is the first time that widespread ALS involvement in the frontal lobe can be visualized using both MR DTI and MRSI imaging studies.

Acknowledgement: This study was supported in part by a grant from National Institute of Neurological Disorders and Stroke (HM/DS, R01-NS41672), MDA Wings Over Wall Street, and ALSA (AU).

C66 COGNITIVE IMPAIRMENT IN PRIMARY LATERAL SCLEROSIS

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Background: Primary lateral sclerosis (PLS) is a rare upper motor neuron disease with adult onset. To date, there has been limited systematic investigation of the neuropsychological features of the disease, and no investigation into its emotional/behavioural features.

Objectives: The goal of the current study was to complete a prospective investigation of the possible cognitive and emotional/behavioural effects of PLS in a relatively large series of patients ($n = 18$).

Methods: Five major domains of neuropsychological functioning were assessed: executive skills; attention/concentration; visual-perceptual/constructional skills; memory; and emotional/behavioural functioning. Tests were chosen to minimize the requirements for speech and

manual motor skills. All scores were converted into T-scores (mean of 50, SD of 10) based on normative samples, corrected when possible for age and education. For the purposes of this study, a T-score less than 40 (less than 1 SD below the mean) was considered an abnormal performance. A patient's overall performance was considered impaired if he/she scored in the abnormal range on two or more measures.

Results: Results indicated a considerable amount of heterogeneity with some patients showing no impairment and others showing significant deficits. Overall, 11 out of 18, or 61% of patients were considered to be cognitively impaired. The areas of cognition most affected were as follows: oral word fluency (50% of subjects impaired); spatial working memory (40% impaired); verbal list learning (33% impaired); and auditory verbal working memory (29% impaired). There was no relationship between cognitive functioning and various disease parameters including disease duration, current site of disease, site of onset, and respiratory variables. Only three patients endorsed significant symptoms of depression. Results of behavioural inventories administered to patients' caregivers indicated abnormal behavioural symptoms (to be described) in a majority of patients.

Discussion and conclusions: 1) Patients with PLS are heterogenous with respect to cognitive impairment and behavioural symptoms; 2) However, the majority have at least mild cognitive impairment, and many have abnormal behavioural symptoms; 3) Deficits are most prominent in the areas of executive functioning, working memory, and learning efficiency; 4) Though not in the range of frontotemporal dementia, behavioural symptoms are common; 5) The findings are consistent with frontal circuitry dysfunction; and 6) The cognitive profile of PLS patients is very similar to that of sporadic ALS. These results suggest that cognitive impairment in PLS and ALS both result from dysfunction of similar neuroanatomical regions.

Acknowledgement: This research was supported by ALSA.

C67 COGNITIVE VULNERABILITY IN SUBGROUPS OF MND

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Background: Traditionally intellect was thought to be preserved intact among patients with MND. However, it is now accepted that MND is associated with frontotemporal dementia (FTD) in 3–5% of sporadic ALS cases, and ~15% of familial ALS cases. In addition, a larger group of patients displays mild cognitive deficits, and these can be detected only by detailed neuropsychological assessment. However, few studies have investigated cognitive change in rarer subgroups of MND to investigate the possibility of differential cerebral vulnerability.

Objectives: To identify the profile of cognitive, behavioural and emotional change in three subgroups of MND patients: sporadic ALS, familial ALS, progressive muscular atrophy (PMA), and a group of matched healthy controls. As a higher proportion of familial ALS cases are associated with FTD it was predicted that they would show higher levels of cognitive and behavioural change. By contrast, as PMA patients lack upper motor neuron (UMN) involvement it was predicted they would perform as well as healthy controls.

Methods: Forty-one sporadic ALS patients, 15 familial ALS patients, 10 PMA patients, and 35 healthy controls matched for age and IQ were recruited. They underwent a range of neuropsychological tests evaluating executive functions, memory, language, and visuospatial perception. Patients and carers completed behavioural self-report questionnaires on aspects of dysexecutive function, behavioural change and emotional lability. Overall group differences were assessed by one-way ANOVAs with post-hoc Bonferroni analyses to identify deficits in MND subgroups relative to controls.

Results: While familial ALS and PMA patients had a longer disease duration, all patients had a similar level of functional disability. Relative to controls, familial ALS patients demonstrated deficits on tests of sentence completion, judgement of line orientation, and higher levels of self-rated executive dysfunction following disease onset. In addition, there was an overall between-groups trend for verbal fluency, accounted for by poorer performance by familial ALS patients relative to controls. Sporadic ALS patients had deficits on tests of confrontation naming and higher levels of emotional lability. PMA patients performed in a manner comparable to the control group.

Conclusions: Results support the hypothesis that familial ALS patients may be more susceptible to cognitive and behavioural change, suggesting a different pattern of cerebral vulnerability from sporadic ALS and PMA patients.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 49–51

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SESSION 8C GENETICS

C68 THE GENETICS OF MOTOR NEURON DISEASE: WHERE ARE WE NOW?

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It has long been accepted that motor neuron disease is either familial or sporadic, but the evidence is that even those with sporadic disease have an underlying genetic susceptibility that caused the motor neurons to be vulnerable. In fact, motor neuron disease is perhaps best thought of as a disease showing so-called complex genetics, in which multiple genetic and environmental factors interact over a lifetime, leading to motor neuron death. Disentangling the different risk factors in such conditions is difficult, but the laboratory techniques, computer power and statistical methods are now available to make this possible. Over the past decade we have moved from a position where the only known genetic cause of motor neuron disease was mutation in the SOD1 gene, to a situation where we now have at least five known motor neuron disease genes, six more loci in which the genes are yet to be identified, and several genes likely to be associated with sporadic ALS. Finding these genes is becoming important not only because each new gene adds another piece to the jigsaw of what causes motor neuron disease, but also because new techniques in gene therapy and RNA silencing may allow different approaches to treatment.

C69 SPASTIN MUTATIONS IN SPORADIC ADULT-ONSET UPPER MOTOR NEURON SYNDROMES

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Background: In patients with an unexplained sporadic upper motor neuron (UMN) syndrome, clinical distinction between primary lateral sclerosis (PLS) and sporadic hereditary spastic paraparesis (HSP) may be problematic. Screening for mutations in known HSP genes offers a new tool to differentiate sporadic HSP from PLS. The single most common cause of pure HSP is mutation of the spastin gene (*SPG4*), representing about 40% of the patients.

Objectives: To investigate whether spastin mutations are present in patients with PLS and sporadic HSP.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045212

Methods: We screened the spastin gene in 99 Dutch patients who were identified after a nationwide search for patients with an unexplained sporadic adult-onset UMN syndrome. Inclusion criteria were a progressive UMN syndrome, adult onset, duration >6 months and negative family history for HSP. Exclusion criteria were clinical evidence of lower motor neuron (LMN) loss (amyotrophy) and evidence of other causes using the following laboratory investigations: serum biochemistry (including TSH, vitamin B12, folate, and vitamin E), very long chain fatty acid analysis in plasma, serology (syphilis, borreliosis, HTLV-1 and HIV), bile alcohol analysis in urine, and cerebral and spinal MRI.

Results: Ninety-nine patients were included in this study. Fifty-two patients had symptomatic arm or bulbar involvement, which would suggest a diagnosis of PLS. The other 47 patients had leg involvement only, consistent with a typical pure HSP phenotype. We found six mutations, of which four were novel, in the subgroup of 47 patients with UMN symptoms restricted to the legs (13%). Another novel spastin mutation was found in a patient with a rapidly progressive tetrapyramidal and pseudobulbar syndrome and subsequent progression to clinically definite ALS.

Conclusions: Our study shows that spastin mutations are a relatively frequent cause of sporadic spastic paraparesis, but are not a cause of PLS. The finding of a spastin mutation in a patient with rapid progression to clinically definite ALS may suggest a role of spastin mutations in the pathogenesis of ALS.

C70 VAPB MUTATIONS IN ALS

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Background: The number of genes identified in adult-onset amyotrophic lateral sclerosis (ALS) families is currently limited, with SOD1 mutations accounting for approximately 15–20% of familial cases. Recently, the gene at the ALS8 locus on chromosome 20q13.33 was found to be the vesicle-associated membrane protein/synaptobrevin associated protein B (*VAPB*) gene. One missense mutation was identified in a Brazilian patient with rapidly-progressing ALS and among other Brazilian families with the same mutation but presenting with spinal muscular atrophy (SMA) or atypical ALS. The

ubiquitously expressed VAPB has a microtubule-associating domain and interacts with membrane vesicles.

Objectives: Our goal was to determine whether VAPB mutations are present in more than one population, along with the frequency of VAPB mutations in both sporadic and familial ALS cases. Understanding the type of mutation involved and the location of mutations in the VAPB protein was also of interest.

Methods: One hundred sporadic and 69 familial samples with definite ALS collected from North America and France were screened. The entire six-exon *VAPB* gene was analysed by direct sequencing, including 50bp into introns. Control individuals were likewise compared by direct sequencing.

Results: A 3 base-pair deletion was identified in exon 5 in a familial ALS patient resulting in the loss of a serine amino acid in the full-length protein (p.160delS). Also in exon 5, an alanine-to-valine missense mutation was identified in a sporadic ALS case (p.A145V). Neither of these changes were found in 192 ethnically-matched control individuals. The mutations arise in reasonably conserved regions of the *VAPB* gene between a coiled-coil domain and the hydrophobic transmembrane region.

Discussion and conclusions: This study shows the presence of additional ALS mutations in the *VAPB* gene. The mutations are observed in a separate population from the initial Brazilian mutation, increasing the overall spectrum of patients who could be affected. Additional work is now needed to determine how these mutations relate to the pathogenesis of the disease.

C71 SEGREGATION OF TAU HAPLOTYPE IN ALS

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Background: Two extended haplotypes have been described across the human *tau* gene (1). The more common H1 haplotype is over-represented in individuals with frontotemporal dementia (FTD), supranuclear palsy, corticobasal degeneration, argyrophylic grain disease and Parkinson's disease (2). The tau CA3662 polymorphism is associated with ALS-Parkinsonism dementia complex of Guam. Given the overlap in histologies between FTD and ALS, the *tau* gene is suspected of being a susceptibility locus in ALS. There is only one published study of tau haplotypes in ALS (3). This study identified a trend towards increased H1 frequency in individuals with ALS (n=108) versus controls (n=168) (77 vs. 73, p=0.22). However, this study may be under-powered and verification of these findings in other populations is currently unavailable.

Objectives: To test the hypothesis that possession of the H1 haplotype confers susceptibility for the development of ALS.

Methods: We characterized the tau haplotypes in 791 individuals with sporadic ALS and 622 ethnically matched controls from three independent populations. Further screening of other populations will be presented.

Results: We identified no significant difference in H1 haplotype frequencies in the ALS group versus controls (0.79 vs. 0.78, p=0.35). The H1 homozygous genotype was over-represented in individuals with sporadic ALS in one population versus controls (0.66 vs. 0.60, p=0.043). Preliminary data has shown association between the H1 homozygous genotype and ALS+FTD. Further sub-population analysis will be presented.

Conclusions: These data suggest that the tau locus is not a significant risk factor for ALS, however the H1 homozygous state may confer susceptibility for ALS+FTD.

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C72 INCREASED INCIDENCE OF THE HFE H63D VARIATION, BUT NOT OF C282Y AND C65S, IN SPORADIC ALS PATIENTS OF ITALIAN ORIGIN

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Background: A role for metal-mediated oxidative stress in the pathogenesis of ALS has been proposed since 1994 in the first studies of FALS mutant SOD1, and an interference with iron homeostasis has been postulated. Elevated iron levels have been described in the spinal cord of ALS patients, leading to the hypothesis that the presence of Fe within spinal motor neurons makes them susceptible to ALS-type degeneration via the production of hydroxyl free radicals from hydrogen peroxide generated by Cu/Zn superoxide dismutase. The HFE gene on chromosome 6 is a MCH class I-like molecule related to iron regulation. Mutations in the coding region cause Hereditary

Hemochromatosis (HH), a common autosomal recessive disorder of iron metabolism that leads to iron overload in adult age. Two recent reports on HFE mutations in ALS lead to conflicting results, with one study describing a prevalence of the HFE mutations in ALS higher than in the control group, and the second with no difference between the ALS patient population and the control group. In the healthy population of Italian origin the overall allele frequency for the C282Y mutation is 0.5%, 12% for the H63D and 1.1% for the S65C mutation.

Objectives: To investigate whether mutations in the HFE gene could represent a risk factor for ALS, we characterized 125 ALS patients and 168 controls matched for age, sex and ethnic origin.

Methods: To test H63D, C282Y and C65S we used the pyrosequencing technique, which is based on sequencing by synthesis and relies on real time quantification of pyrophosphate release during DNA synthesis.

Results: In the current study, 31.2% of sporadic ALS patients carried a mutation in the HFE gene (24.8% are heterozygous for H63D, 2.4% are homozygous for H63D, 1.6% are heterozygous for C282Y and 2.4% are heterozygous for S65C), compared to controls in which the heterozygous for H63D are 15.6%, the heterozygous for C282Y are 1.2%, and for S65C are 0.6%.

Discussion: The role for disrupted iron metabolism is suggested from many recent reports; in this study we investigated the variations H63, C282Y and S65C in the HFE gene in 125 sporadic ALS patients and in 168 matched control individuals from Italy, and found differences in the H63D variant at the $p < 0.004$ level of significance. The odds ratio for the H63D variant was 2.199. These data demonstrate a significant association of ALS and H63D variation in the HFE gene in an Italian population, supporting the hypothesis that the alteration of iron metabolism may confer susceptibility to neurodegenerative diseases such as ALS.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 52–54

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SESSION 9A MITOCHONDRIA: BIOENERGETICS AND CELL DEATH

C73 A MITOCHONDRIAL PARADIGM FOR AGE-RELATED METABOLIC AND DEGENERATIVE DISEASES

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The mitochondrial (mt) genome encompasses approximated 1500 genes, 37 encoded by the maternally-inherited mtDNA, which is present in thousands of copies per cell, and the remainder encoded by various chromosomal loci. The mitochondria produce most of the cellular energy, generate much of the endogenous reactive oxygen species (ROS) as a toxic by-product, and can initiate apoptosis via activation of the mitochondrial permeability transition pore (mtPTP): when energy production declines, oxidative stress becomes excessive, and calcium accumulates in the cell and mitochondria. Because of its direct exposure to ROS, the mtDNA has a very high mutation rate resulting in the age-related accumulation of somatic mtDNA mutations in post-mitotic tissues. As mtDNA mutations accumulate, mitochondrial energy production declines until the mtPTP is activated and the cell with its defective mitochondria is destroyed. Therefore, the accumulation of somatic mtDNA mutations is the aging clock. Age-related diseases result from the combined effects of an inherited predisposition to the disease, environmental insults, and the accumulating somatic mtDNA mutations. When these combined effects exceed the threshold of the mtPTP, cells are lost. When sufficient cells are lost within a tissue, clinical symptoms ensue.

C74 REGULATION OF MITOCHONDRIAL AND ER CELL DEATH PATHWAYS

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The activation of genetically and biochemically determined cell death programs are believed to contribute to pathophysiological cell death in acute and chronic neurological disorders. The identification of the individual genes and proteins mediating neuronal cell death in response to different stress conditions is therefore of major interest for current neuroscience research. The Bcl-2 family proteins are key regulators of these cell death programs. They have been shown to be involved in the control of caspase-dependent and -independent apoptosis. Caspase-dependent apoptosis results from an increase in mitochondrial outer membrane permeability. It leads to the release of cytochrome C from mitochondria and the formation of a caspase-activating multiprotein complex, the apoptosome. Caspase-independent apoptosis can result from the mitochondrial release of a pro-apoptotic nuclease, the apoptosis-inducing factor (AIF). Caspase-independent cell death may also occur through mitochondrial dysfunction secondary to the loss of cytochrome C, and has been shown to involve energy depletion, increased ROS production, and the activation of autophagy. Bcl-2 and related anti-apoptotic proteins such as Bcl-xL protect neurons against caspase-dependent and -independent cell death pathways by inhibiting the activation of pro-apoptotic Bcl-2 family members. Bax and Bak are pro-apoptotic Bcl-2 family members that are able to form a megachannel in the outer mitochondrial membrane large enough for the permeation of proteins such as cytochrome-C. In order to cause this permeability increase, Bax and Bak undergo a conformational change and insert into the outer mitochondrial membrane.

In apoptotic cells, the transcriptional induction or post-translational activation of Bcl-2-homolgy domain-3 (BH3)-only proteins triggers the activation of Bax and Bak. BH3-only proteins either directly activate Bax and Bak, or interact with and neutralize the anti-apoptotic activity of Bcl-2 and Bcl-xL. BH3-only proteins are structurally diverse and couple specific upstream stress signals to the evolutionary conserved mitochondrial apoptosis pathways. BH3-only proteins can be activated via transcriptional induction (PUMA, Noxa, Hrk, Bim, BNIP3L), phosphorylation (Bad, Bik, Bim, Bmf), or proteolytic cleavage (Bid). In the nervous system, induction of PUMA occurs in response to p53 activation and as a consequence of prolonged ER stress, while induction of BNIP3L occurs during hypoxia. Activation of Bim has been implicated in trophic factor withdrawal-induced apoptosis in neurons, as well as in response to the activation of stress-activated protein kinases. Finally, Bid has been shown to be required for excitotoxic and ischaemic nerve cell injury. Both Bcl-2 and Bcl-xL reside in the outer mitochondrial membrane, but are also localized to the ER membrane and nuclear envelope, facing the cytosol. Evidence has been presented that exclusive ER localization of Bcl-2 is sufficient to protect cells against several apoptosis stimuli, possibly by sequestering pro-apoptotic family members at the ER.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045221

C75 IMPAIRED MITOCHONDRIAL ANTI-OXIDANT DEFENCE IN SOD1-RELATED FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background: Mutations to Cu/Zn superoxide dismutase (SOD1) are responsible for ~2% of amyotrophic lateral sclerosis (ALS). It remains unclear how mutant SOD1 injures motor neurons but increasing evidence suggests that mitochondrial dysfunction may be important in the pathogenesis of both the sporadic and familial forms of the disease.

Objectives: 1) To identify changes in mitochondrial protein expression attributable to the presence of mutant SOD1 in a motor neuronal cell-culture model of SOD1-related familial ALS; 2) To establish whether these changes in protein expression have functional consequences and demonstrate relevance to ALS in patients.

Methods: 2D-SDS PAGE, MALDI-TOF MS and online database searching were used to identify changes in mitochondrial protein expression brought about by the presence of mutant SOD1 in NSC34 cells. Confirmatory Western blotting was performed in NSC34 cells and key changes also confirmed in mitochondrial preparations of SOD1 transgenic mouse spinal cord. Q-PCR of laser-capture micro-dissected spinal motor neurons from sporadic ALS and SOD1-related FALS cases was then used to compare the mRNA levels of key protein changes with those of controls.

Results: The expression of 29 proteins changed in a mutant SOD1-specific manner. These included anti-oxidant enzymes, apoptotic effectors, and electron transport chain components. Peroxiredoxin 3 (Prx3), a thioredoxin-dependent hydroperoxidase, was down-regulated in mutant SOD1-expressing cells and in SOD1 transgenic mouse spinal cord. Immunocytochemistry confirmed mitochondrial expression of Prx3 in NSC34 cells and immunohistochemistry was used to confirm expression of Prx3 within murine and human spinal motor neurons. Q-PCR for Prx3 further suggested down-regulation in SALS and SOD1-related FALS cases compared to controls. Data from the pharmacological manipulation of mitochondrial anti-oxidant defence arising from this work will also be presented.

Conclusions: Given the evidence for oxidative stress in ALS, it is interesting that Prx3, an anti-oxidant mitochondrial matrix protein, is down-regulated in the presence of mutant SOD1 in NSC34 cells, in SOD1 transgenic mice and in patients. Changes in mitochondrial anti-oxidant defence may play a role in the death of motor neurons in SOD1-related FALS and its modulation may offer therapeutic opportunities.

C76 CNS METABOLIC DEFECTS PRECEDE PATHOLOGIC ALTERATIONS IN A MOUSE MODEL OF FAMILIAL ALS

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Background: Multiple cell death pathways have been implicated in the selective loss of motor neurons in ALS, but the causal event remains enigmatic. One hypothesis implicates metabolic dysfunction, since alterations in energy metabolism and mitochondrial function occur in patients. In addition, expression of mutant Cu/Zn-superoxide dismutase (SOD1), linked with ~25% of familial ALS (FALS), can induce mitochondrial abnormalities.

Objectives: To determine if metabolic defects contribute to disease onset *in vivo*, we examined the association between CNS energetic defects and disease progression in G93A mutant SOD1 mice.

Methods: We used quantitative [^{14}C]-2-deoxyglucose autoradiography to measure glucose use rates in nine spinal cord and 49 brain regions of conscious pre-symptomatic (60-day) and symptomatic (120-day) G93A mice, in age-matched wild-type littermates, and in aged N1029 mice overexpressing human wild-type SOD1. We also determined metabolite levels by HPLC in flash-frozen brain and spinal cord tissue from G93A and wild-type mice at multiple ages, in G93A mice treated with creatine (2% in diet) from symptom onset, and in aged N1029 SOD1 mice.

Results: Glucose use rates were impaired in multiple brain components of the motor system in G93A mice as early as 60 days of age, preceding the first detectable pathologic changes (~70–80 days) and onset of hind-limb weakness (~90–100 days). At 60 days, glucose use was reduced in components of the corticospinal projection, notably primary motor cortex (Fr1), and several areas synaptically associated with Fr1 including the pontine nuclei and reticular formation of the bulbospinal pathway, and some thalamic nuclei. In the spinal cord, regarded as the crucial site of neuronal dysfunction in ALS, glucose metabolism was unaltered at 60 days, but was markedly impaired in cervical and thoracic grey matter by 120 days. Aged (21-month-old) N1029 mice showed no alterations in cerebral or spinal cord glucose use, implying that the changes detected in G93A mice are due to the SOD1 mutation rather than overexpression. HPLC revealed significant depletions in ATP levels in the cerebral cortex of G93A mice evident as early as 30 days of age, implying that reduced neuronal energy generation is an extremely early consequence of mSOD1 expression. Alterations in spinal cord did not reach

significance. ATP depletion was partially rescued by creatine administration.

Conclusion: In conclusion, these studies demonstrate that energetic defects occur earlier than other pathogenic processes reported in G93A mice, and suggest that dysfunction within the corticospinal projection may precede alterations in spinal neurons in this FALS model. Overall, results support a critical role for metabolic dysfunction in the pathogenesis of ALS.

Amyotrophic Lateral Sclerosis. 2005 (1); 6: 55–56

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SESSION 9B POPULATION GENETICS AND EPIDEMIOLOGY

C77 AN INTRODUCTION TO COMPLEX GENETICS: PITFALLS IN IDENTIFYING DISEASE SUSCEPTIBILITY IN SPORADIC POPULATIONS

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No abstract available.

C78 EPIDEMIOLOGY AND ETHNIC DIVERSITY IN ALS

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It is generally stated that the incidence of ALS is the same throughout the world; however, there have been no well-conducted comparative studies of ALS occurrence among different populations, ethnic groups or defined geographic areas. Epidemiological studies in regions that comprise non-Europeans have generally yielded lower figures. An ethnic difference in susceptibility to ALS has been invoked to explain the low rate of ALS in Mexico, but restricted access to medical care among lower socio-economic groups may also account for this finding. Similarly, small epidemiological studies in Rochester county, Minnesota, have reported variation in the presentation of ALS in different ethnic groups. More recently, Kasarskis et al. demonstrated a risk ratio for ALS of 0.61 in Blacks vs. Whites, and of 2.08 for Hispanics, Asians and Others vs. Whites, in a cohort drawn from veterans of the first Gulf war. Population-based epidemiological studies from Asia are limited.

Mortality studies also suggest differences based on ethnicity. A study of mortality from ALS revealed lower rates among UK immigrants from the Indian subcontinent, the Caribbean, and Africa compared with the general population in England. More recent mortality data from the southern US also suggests a lower incidence of ALS in African-Americans.

ALS is increasingly recognized as a complex genetic disease. Variations in phenotype have long been recognized, some of which correlate with specific Mendelian mediated genotypes (e.g. the autosomal recessive Scandinavian D90A mutation in SOD1). It is therefore likely that ethnic background may be important in determining the relationship between environmental risk factors and genetic susceptibility. The conflicting reports of the relative risks of various susceptibility genes including ApoE4, VEGF, SMN1 etc. may in part be due to population-based differences. Indeed, the carrier frequency of SMN1 differs considerably among different ethnic groups, as evidenced by a recent population-based study in Cuba.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045230

However, it does not always follow that genetic factors necessarily underlie variations in risk between ethnic groups. Studies in Guam have implicated both genetic and environmental factors that may underlie the high risk in this group.

The definitive strategy for distinguishing genetic and environmental explanations for ethnic differences in disease risk is to study populations where there has been admixture between low-risk and high-risk groups.

A population-based epidemiological study of ALS is ongoing in Cuba. The Cuban population is ethnically diverse and genetically admixed. Access to healthcare in Cuba is universal, and population-based epidemiology is feasible as a result. Using a panel of recently developed ethnicity specific polymorphic markers it will be possible to test the hypothesis that ALS occurs less frequently in populations of African ancestry than in those of European (mainly Spanish) ancestry.

If the epidemiological observations are correct that ALS is less common in those of African descent, modern bioinformatics technology can be used to identify both 'at risk' and 'protective' genotypes that will help to elucidate disease pathogenesis.

C79 DIFFERENT DOWN UNDER? MOTOR NEURON DISEASE IN THE SOUTHERN HEMISPHERE

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Background: Studies of large numbers of people with MND have so far been restricted to the northern hemisphere. Epidemiological studies of disorders such as schizophrenia suggest that environmental influences on diseases can differ between the hemispheres. On the other hand, southern hemisphere data can be useful to support that of northern hemisphere studies. An island continent such as Australia is an ideal site to look for environmental factors underlying MND.

Objectives: To set up a database in Australia to enable a southern hemisphere study of environmental and genetic factors in sporadic MND.

Methods: An epidemiological and DNA database for MND was set up in Australia in 2000. Cases are recruited via motor neuron disease associations in all mainland states of Australia. The investigator travels to each state capital on a regular basis to collect blood samples from subjects. Controls are spouses, friends and relatives of MND cases. All subjects complete an eight-page questionnaire which includes items such as ethnicity, lifetime

residences and travel, employment, exposure to chemical or toxins, injuries and personal habits.

Results: So far over 900 subjects have given questionnaire data and blood samples to the Bank. One-third have MND (70% with ALS) and two-thirds are controls. Risk factors we are examining are parental age and number of siblings (no difference), season of birth (no difference), pesticide exposure (slightly increased in ALS) and subject and parent smoking (in progress). We have undertaken case-control association studies on susceptibility genes for heavy metals (metallothioneins) (1), enteroviral infection (the poliovirus and other enteroviral receptors) and organophosphate toxicity (paraoxonase). The poliovirus receptor (2) and paraoxonase genes show differences in functional polymorphisms in ALS, suggesting that susceptibilities to viruses or toxins could underlie some cases.

Discussion and conclusion: This is the first epidemiological and DNA Bank in the southern hemisphere, and the first to cover an entire continent. The Bank holds the potential to uncover environmental risk factors and to look for gene-environment interactions in MND.

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C80 STRUCTURED INTERVIEW FOR GENETIC AND ENVIRONMENTAL RISK FACTORS IN ALS AND ITS TEST-RETEST RELIABILITY EVALUATION

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Background: In ALS, accurate identification of environmental exposures based on recall is a difficult but crucial methodological issue. In-person interview is the gold standard in environmental epidemiology, but can be expensive, limits access to cases, and possibly reduces participation rates. Development of a reliable structured interview that can be administered by telephone, could improve efficiency and maximize participation rates in future studies.

Objectives: The first objective was to develop a structured interview for identifying environmental exposures and family history of neurological disorders in patients with ALS. The second objective was to evaluate the test-retest reliability of the instrument, and specifically to assess agreement between in-person and telephone interviews.

Methods: A structured interview instrument was developed based on literature review, a consensus obtained at an international advisory board meeting (held in February 2004), our previous experience in ALS epidemiology, and input from an environmental hygienist (LA). Test-retest reliability was assessed by administering two interviews to each of 30 randomly selected patients with ALS from a tertiary referral center and 30 controls (mostly family members). Each subject was assigned to one of three interview modalities: in-person to in-person, in-person to telephone or reversed, and telephone to telephone. First and second interviews were performed within two weeks. Kappa (a measure of agreement with desirable properties) was calculated for each interview item.

Results: The major interview domains consisted of: disease information and demographics (30 items); family composition (63); family medical history (152); lifetime residential history (29); lifetime occupational history (85); military service (54); hobbies and leisure time activities (108); lifetime exercise and sports activity (53); and tobacco and alcohol (25). The duration of the entire interview averaged approximately 2 h (range 1–3 h). The number of case-control pairs assigned to each interview modality was 3 for in-person to in-person, 10 for in-person to telephone, 10 for telephone to in-person, and 7 for telephone to telephone. Kappas were greater than 0.8 for most items, but were lower for some exercise items before the age of 17 years, as well as for detailed residential and occupational recalls. These sections of the interview have been streamlined based on this experience. Kappas for pairs involving a telephone and an in-person interview were not substantially lower than for other pairs. Kappas for cases did not differ markedly from those for controls.

Discussion and conclusions: These results indicate good test-retest reliability for most items included in the instrument, suggesting that it will be useful for future epidemiologic studies. The good agreement between in-person and telephone interviews suggests that telephone administration of this interview can be used in future epidemiologic studies in ALS.

Acknowledgement
We gratefully acknowledge the generous support from MDA Research Grant and the MDA Wings Over Wall Street Fund.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 57–59

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SESSION 10A ANIMAL MODELS

C81 MICE: HOW THEY HELP US STUDY MOTOR NEURON DEGENERATION

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Mice have long been used in the study of basic biology and as models for understanding the pathology of human disease, and in first trials for treatments. However, there are many different types of mouse model, each relevant to different types of study, each with specific pros and cons. In ALS research the SOD1 transgenics are of paramount importance, and they are now joined by several recent models that have modifications of other genes, all of which are adding in to the big picture of what causes motor neuron death. New data are also coming from mice in which we do not yet know the causal genetic mutation.

As with all animal studies we have to carefully evaluate biomedical research needs and what society finds acceptable in the level of distress to individual mice. We also need to be clear about what our phenotype testing is really telling us, as while mice share almost the same biochemistry and cell biological pathways as humans, our physiology can be different.

Overall many mouse models, especially the SOD1 transgenics, are providing key information in the study of motor neurons in health and disease, and in the search for effective therapies.

C82 IDENTIFICATION OF MODIFIER GENES THAT CAN DELAY DISEASE ONSET IN A MOUSE MODEL FOR ALS USING GENETIC MAPPING AND GENE EXPRESSION PROFILING

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Background: Mice carrying mutated SOD1 in a FVB genetic background are widely used as a model to study ALS. They normally develop symptoms resembling ALS and die between 90 and 120 days of age. When these mice are crossed with mice from the C57B16/129Sv background, the offspring develop a delayed ALS onset, ranging from 140 days to 2 years, despite the presence of mutated SOD1 (1). This indicates that the expression of the ALS phenotype depends upon the genetic background and suggests that certain genes can delay the toxic effects of mutated SOD1.

Objective: To identify modifier genes that can delay disease onset in a mouse model for ALS using genetic mapping and gene expression profiling.

Methods: For the identification of the genes that can delay disease onset as a result of the mixed genetic background, we use a combination of gene expression analysis, genotype differences and clinical data (the 'genetical genomics' approach). Here, mRNA transcripts are treated as quantitative traits and correlated with DNA marker information. This hopefully points toward specific regions on the genome responsible for differential gene expression (expression QTL) which may lead to the identification of candidate genes responsible for delayed disease onset.

Results: Mice with mixed background and delayed onset were genotyped previously using nearly 200 genomewide polymorphic markers, which linked several loci to the delayed disease phenotype (1). So far we have isolated and amplified RNA from spinal cord tissue. Samples were labeled with either Cy3 or Cy5 and hybridized to oligo microarrays containing over 32,000 mouse-specific genes and splice variants using an extended loop design. Linking transcript abundance to the genetic markers is in progress.

Discussion: Results will be presented at the meeting. It is hoped that this study identifies genes involved in delayed disease onset that may be targets for future treatment of ALS.

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C83 THE ROLE OF ALSIN IN JUVENILE ONSET ALS

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Background: Mutations in the *ALS2* gene, encoding alsin, cause autosomal recessive, juvenile onset ALS (ALS2) and related conditions. The nine mutations that have been detected thus far are all predicted to lead to premature truncation of the alsin protein and a complete

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045249

loss of function. The intact alsin protein has been shown to act as a guanine nucleotide exchange factor (GEF), or activator, of the GTPases Rab5 and Rac1. This suggests a role for alsin in endocytosis and/or vesicle trafficking.

Objectives: We are seeking an understanding of the function of alsin and its role in ALS2 pathogenesis by studying its detailed expression pattern, and by the generation and analysis of a mouse model of the human disease.

Methods: To study the expression of alsin we generated two new reagents: 1) a highly specific monoclonal antibody (N-alsin 24), directed towards the N-terminus of alsin; and 2) transgenic mice expressing the β-*gal* gene under the control of the ALS2 promoter, enabling *in-vivo* visualization by lacZ staining of the cell types in which ALS2 is expressed. The β-*gal* gene in these mice replaced exons 3 and 4 of the normal gene, resulting in a null allele, thereby concurrently generating a model of ALS2.

Results and conclusions: In the adult mouse, alsin is predominantly expressed in the CNS, with high levels in the cerebellum, choroid plexus and alpha motor neurons of the spinal cord. Alsin expression colocalized with the neuronal marker NeuN but not the glial marker GFAP. In the cerebellum, the alsin protein appears to reside in the axons (but not the cell bodies) of the granular cells, which make up the bulk of the molecular layer. Expression was not observed in the Purkinje cells. Other areas of the brain showed moderate expression (including hypothalamus, amygdala and hippocampus) (1). In the periphery, expression was detected in testis and kidney, and weakly in heart and liver. Alsin was expressed throughout mouse development, although primarily in the periphery. CNS expression became predominant in neonates, largely reflecting the postnatal development of the cerebellum (1). ALS2 null mice are viable and are born at the expected Mendelian frequency. They are fertile and exhibit no gross abnormalities. In later life, they exhibit mild behavioural deficits and neuropathological changes. These mice, and cells derived from them, will prove useful in future studies of ALS2 pathogenesis.

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C84 DEVELOPMENT OF AN ALSIN KNOCKOUT MOUSE MODEL

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Background: ALS2 is characterized by bilateral pyramidal syndrome, weakness with atrophy and fasciculation of the hands and/or legs without sensory disturbance. ALS2 has an early onset at an infantile and a juvenile age with very slow progression. We have previously identified a novel gene named alsin, mutations in which cause juvenile ALS type 3 (ALS2) or juvenile primary lateral sclerosis (JPLS) depending on the location of the mutations. All the mutations identified in alsin so far lead to truncated alsin protein. Thus it is postulated that loss of normal function of alsin leads to motor neuron degeneration in ALS2. Alsin is relatively a large gene with 83kb genomic DNA in size. It has 34 exons with the first exon being non-coding. Alsin has two transcriptional forms with two distinct poly(A) signals and encodes one short and one long form of protein product, alsin. The short form of alsin gene has four exons, encoding 396 amino acids (aa). The long form of alsin has 34 exons, encoding 1657aa. Both forms share the first four exons. The physiological function and the pathogenic mechanism underlying ALS2 are not known.

Objective: To investigate the physiological function of alsin and the pathogenesis of ALS2.

Methods: We constructed a targeting vector designed to replace exon 4 and a part of exon 3 with neo-cassette. This vector was designed to target both short and long forms of alsin, leading to a very short, truncated polypeptide consisting of only nine amino acids. We selected two positive ES cells. We developed alsin knockout mice from these ES cells.

Results: The alsin knockout mice show normal lifespan with mild deficit in motor function on RotaRod test. Degenerative pathology is mainly observed in the corticospinal tracts in the dorsal column of the spinal cord. No apparent motor neuron pathology was found in the cortex, brain stem and spinal cord. We also crossbred the alsin knockout mice with SOD1G93A transgenic mice to generate SOD1G93A transgenic mice on the null alsin background. We found that the disease course of the SOD1G93A mice without alsin is not changed.

Conclusion: Our findings from alsin knockout mouse model suggest that ALS2 in mice is predominantly an axonopathy of corticospinal tracts; loss of alsin is not the pathogenic mechanism underlying ALS1; although motor neuron degeneration is a shared late consequence in both ALS1 and ALS2, the upstream signaling pathways triggering motor neuron degeneration in ALS1 and ALS2 are independent.

C85 MOTOR CO-ORDINATION AND LEARNING DEFICITS, INCREASED ANXIETY, AND SUSCEPTIBILITY TO OXIDATIVE STRESS IN MICE LACKING ALS2

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Background: Mutations in ALS2 have been linked to autosomal recessive juvenile onset amyotrophic lateral sclerosis (ALS2). With the exception of the Tunisian mutation in ALS2 suggested to be associated with both

upper and lower motor neuron defects, at least eight other mutations in ALS2 have been identified and all cause the juvenile or infantile-onset motor neuron disease, particularly affecting upper motor neurons. This Tunisian mutation, a single nucleotide deletion in exon 3 resulting in a premature stop codon, probably abrogates all the potential functions of alsin (the protein encoded by ALS2), including activities from its guanine-nucleotide-exchange factor (GEF) domains.

Objective: To study the physiological role of ALS2 and to clarify the pathogenic mechanisms of ALS2-linked disease.

Methods: To investigate the pathogenic mechanisms of ALS2, we generated ALS2 knockout ($ALS2^{-/-}$) mice. A series of mouse behavioural tests, particularly on its motor functions, was applied. Electrophysiological studies were used to study the action potential transduction in both of periphery motor and sensory nerves. Histological and immunohistological studies were employed to study the neuropathological abnormalities of these animals. Primary cortical cultures were used to study the viability of neurons under oxidative stress.

Results: While $ALS2^{-/-}$ mice develop normally, they exhibit age-dependent deficits in motor coordination and motor learning. Moreover, $ALS2^{-/-}$ mice show a higher level of anxiety as judged by either the open field or elevated plus maze tasks. Although they have not yet developed clinical or neuropathological abnormalities consistent with lower motor neuron disease by 20 months of age, $ALS2^{-/-}$ mice or primary cultured neurons derived from these mice were more susceptible to oxidative stress compared to wild-type controls.

Discussion and conclusion: Taken together with findings that the majority of mutations in ALS2 are linked to upper motor neuron diseases, such as primary lateral sclerosis (PLS) and infantile onset ascending hereditary spastic paralysis (HSP), our observations are consistent with the view that loss of ALS2 is not sufficient to cause lower motor neuron disease and raise the possibility as to whether ALS2-linked mutation found in the Tunisian family should be classified as part of a spectrum of HSP.

C86 ZEBRAFISH AS A MODEL FOR MOTOR NEURON DISEASES

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Animal models are a vital resource for studying human disease with each model organism offering different strengths. The zebrafish is both a genetic model system and is superbly suited for developmental biology studies. These attributes have resulted in it increasingly being used to model human diseases. Models generated by both forward genetics; isolating mutations that affect development of a particular organ or tissue, and by reverse genetics; using targeted gene knockdown or scanning genes in mutagenized fish to find gene-specific mutations, have been successful at uncovering novel aspects of human diseases. For diseases not caused by gene deletion but by expression of a mutant form of a gene, transgenic fish can be generated. Once the disease model is established, a number of powerful techniques can be used to analyze the biological basis of the disease. In the context of motor neuron diseases, transgenic fish with GFP-expressing motor neurons can be used to visualize motor axon outgrowth, genetic mosaics can be generated to determine the contribution of specific cell types to the disease process, and electrophysiological and behavioral analysis can be applied to examine the consequences of the lesions. Furthermore, it is also possible to perform suppressor screens to reveal genetic pathways or drug screens to identify therapeutic agents.

The biological basis of two of the most common motor neuron diseases, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), are not well understood thus hindering development of therapeutic strategies. Due to their external development and stereotyped neuromuscular system, zebrafish offer an excellent opportunity to address the biological basis of these diseases. SMA is an autosomal recessive disease and the number one genetic cause of infant and toddler mortality. Low levels of the ubiquitous Survival Motor Neuron (SMN) protein cause SMA; it is unclear, however, how decreased SMN protein causes motor neuron cell death. Because low levels of SMN cause SMA, we modeled this disease in zebrafish using morpholino (MO) mediated protein knock down. The earliest phenotype associated with low SMN levels in zebrafish is aberrant motor axon outgrowth. Motor axons innervating the fin and axial muscle stall inappropriately and are excessively branched in animals with decreased SMN. By injecting morpholino into single motor neurons, we can recapitulate these defects indicating that SMN is needed cell-autonomously for correct motor axon outgrowth. By following *smn* MO fish over time, we find a strong correlation between the severity of the motor nerve defects and decreased longevity. This, and data from others showing that SMN is transported down axons to growth cones, indicate an important role for SMN in axon development and suggest that SMA is a motor axon disease. We have more recently begun to model ALS in zebrafish by generating transgenic lines carrying SOD1 mutations. By taking advantage of the strengths of the zebrafish; genetics, accessible development, embryonic manipulations, and trangenesis, we hope to contribute to the understanding of the biological basis of both of these motor neuron diseases.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 60–63

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SESSION 10B RESPIRATORY MANAGEMENT

C87 NEW USES FOR 'OLD BAGS': MULTIDISCIPLINARY RESPIRATORY CARE IN PATIENTS WITH ALS

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This presentation will discuss the multidisciplinary respiratory care we provide to ALS patients in the Rehabilitation Centre in Ottawa, Canada. This approach to care has been shown to improve mortality in ALS and, in particular, attention must be paid to respiratory complications. Together with respiratory therapy and nursing, the respiratory physician evaluates every patient from the ALS Clinic in the Rehabilitation Centre. All patients undergo a careful respiratory history to exclude conditions such as COPD that may contraindicate some of the preventive therapies. Symptoms of dyspnoea, dysphagia, inadequate cough and sleep disordered breathing are discussed. The patients and family are informed about advance directives and ventilatory options are discussed. Since 1995 all patients, within their capabilities, have undergone neuromuscular primary function tests (PFTs). These include; vital capacity, maximum voluntary ventilation, maximum inspiratory and expiratory pressures, spontaneous peak cough flows (PCF_{sp}) and whenever bulbar function allows, PCF with lung volume recruitment (LVR) (PCF_{LVR}) and with LVR added to an abdominal assist ($PCF_{LVR\ ASSIST}$). Patients and families are trained by RTs and physiotherapists to perform LVR with a resuscitation bag and assisted coughing. Clinical follow-up and PFTs are performed regularly with attention to symptoms of apnoea, orthopnoea and cough effectiveness. All patients are informed about invasive and non-invasive ventilation alternatives and each patient who believes they may choose ventilatory support is invited to participate in a hands-on Ventilation Education session. A three-stage, entirely outpatient, education programme is provided with two or three patients, caregivers with an RT.

Discussion includes topics of Volume and Bilevel ventilation, bulbar impairment, secretion management including the CoughAssist^{TM} and consideration of advance directives. Demonstration of different forms of ventilation is provided. When clinically indicated a second visit provides a one-on-one trial of volume or bilevel ventilation and a broad choice of non-invasive interfaces. Within a month, ventilatory equipment is provided on an elective, outpatient basis from a centralized provincial Ventilator Pool and a third final adjustment of comfortable ventilatory parameters is completed. The process of Ventilation Education on knowledge, decision-making and anxiety (Affect) has been evaluated and no negative effect has been observed in emotional state as a result of the education process. As a result of Ventilation Education the number of patients uncertain about their decision fell from 75% to 4% and no patient received invasive ventilation who decided against it after education. Significant knowledge was gained as a result of the Ventilation Education. The provision of early hands-on Education, secretion clearance techniques and non-invasive ventilatory support has not increased the number of patients on tracheostomy ventilation, but a number of patients have managed 24 h non-invasive ventilation using (bilevel) nocturnal mask and daytime (volume) mouthpiece for years.

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045258

C88 PREDICTORS OF NON-INVASIVE VENTILATION TOLERANCE IN PATIENTS WITH ALS

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Background: Non-invasive ventilation (NIV) appears to improve ALS patient survival and quality of life, but little is known about predictors of NIV tolerance. Some studies have shown that ALS patients with more bulbar symptoms are less likely to be tolerant to NIV (1), while others report no difference in tolerability between bulbar and limb onset (2).

Objectives: The purpose of the present study was to determine whether initial clinical features and pulmonary function tests are useful in predicting NIV tolerance among ALS patients.

Methods: Patients diagnosed with definite or probable ALS who were followed in our ALS clinic until death between 2000 and 2003 were included. Patients were prescribed NIV when respiratory symptoms were present or forced vital capacity (FVC) $<50\%$. A retrospective chart review was used to classify patients as tolerant to NIV if used >4 h nightly, documented at each clinic visit. The relationship between tolerance and 1) symptom onset site and 2) FVC were investigated with a χ^2 and Wilcoxon rank sum test, respectively. Multivariable logistic regression was used to investigate the relationship between tolerance and: age, time from symptom onset to NIV initiation, symptom onset location (bulbar versus limb), gender, and FVC at NIV initiation.

Results: There were 139 patients identified. Overall, 72 (52%) were prescribed NIV. There were no differences in prescribing of NIV by gender ($p=0.44$) or site of symptom onset ($p=0.58$). Of those who were prescribed NIV, information on tolerance was available on 50 (70%). Thirty-six (72%) were tolerant to NIV, and 14 (28%) were not. Patients who were tolerant were more likely to have limb onset symptoms ($p=0.01$), and have higher FVCs at NIV initiation ($p=0.04$). In the multivariable model, only

limb onset was independently associated with NIV tolerance (OR=6.25 [1.09, 33.33]).

Discussion and conclusions: These findings indicate that many ALS patients (in this study 70%) are tolerant to NIV. Assessment of predictors of NIV tolerance only identified limb onset symptoms as an independent predictor, although higher FVC at NIV initiation may also be predictive of NIV tolerance. Importantly, duration of disease and age were not predictors of tolerability and should not be considered reasons to withhold NIV.

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C89 EARLIER USE OF NON-INVASIVE VENTILATION MAY PROLONG SURVIVAL IN SUBJECTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Patients with ALS develop weakness of the respiratory muscles and frequently die from respiratory complications. Non-invasive positive pressure ventilation (NPPV) has been shown to prolong survival in ALS when it is used in patients with advanced respiratory muscle weakness. There is evidence that NPPV may slow the decline of vital capacity in ALS, suggesting that earlier use of NPPV may be beneficial. While expert panels recommend using NPPV when a patient's forced vital capacity (FVC) is below 50% predicted, the ideal time to start NPPV remains unknown.

Objectives: To determine whether initiating NPPV when the FVC is closer to normal improves survival over later initiation of NPPV.

Methods: A retrospective cohort study of patients with ALS referred for pulmonary evaluation was performed at a single institution. Subjects were included if they used NPPV and had spirometry performed within three months of starting NPPV. 'Early' use of NPPV was considered initiating when the FVC was $>65\%$ predicted. 'Early' users of NPPV were started on NPPV for abnormal maximal inspiratory pressure or elevated CO_2. Mortality was assessed from medical records, obituaries, social security records and phone interviews with surviving family members. Continuous variables were compared with *t*-tests or χ^2 testing. Survival was compared using the Kaplan-Meier method with Wilcoxon tests.

Results: Sixty patients were followed for a median of 3.5 years from the onset of symptoms. Twelve (21%) of subjects began using NPPV early in the disease course. Early NPPV users were more likely to be male compared to the Standard Use group (91.7% vs. 60%, $p=0.039$). In other respects the two groups were similar: age 54.3 years vs. 56.5 years, time from symptom onset to diagnosis was 1.75 years vs. 1.25 years, ALS Functional Rating Scale was 35.8 vs. 32.2. Ninety-two percent of the Early group had limb onset disease compared to 82.6% of the Standard group. Seventy-five percent of the Early group used riluzole and 84.1% of the Standard group did. While a higher percentage in the Early group (83%) reported using NPPV for 4 or more hours per day than in the Standard group (65%), this was not statistically different ($p=0.443$). Median survival from time of diagnosis was 3.17 years (range 1.8–5.3) in the Early NPPV group and 2.14 years (range 0.6–10.3) in the Standard care group. This difference bordered on statistical significance ($p=0.06$).

Conclusions: Patients with ALS who started using NPPV when their forced vital capacity was greater than 65% of predicted survived approximately one year longer than those who started NPPV with lower FVCs. This suggests that NPPV use may have effects on the respiratory system beyond simply supporting failed muscles. NPPV may result in benefits by resting fatigued respiratory muscles, improving lung compliance, or reducing hypercarbia/acidosis which can impair muscle contractility. Current efforts are underway to validate these findings in a larger group of patients.

C90 PROGNOSIS OF ALS WITH RESPIRATORY ONSET

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Background: Respiratory muscle involvement is a recognized, but often late, complication of amyotrophic lateral sclerosis. A number of case reports have documented that respiratory failure can be an uncommon presentation of patients with ALS. These case reports tend to portray a poor prognosis for respiratory onset ALS, as the case reports often report acute respiratory failure requiring invasive ventilation.

Objectives: To describe the clinical features and prognosis of patients with ALS with respiratory onset.

Methods: We present a case series of 21 patients of ALS with respiratory onset, obtained by searching our ALS database from 1990 to 2005 by symptom of onset. A retrospective chart review extracted information on demographics, age of onset, initial symptoms, progression of symptoms, ventilation, rate of respiratory decline, and time of death.

Results: The group consisted of 17 males and four females with a mean age of onset of 65 ± 7 years. Three of the patients are still living. The most frequent respiratory

symptoms reported were increasing shortness of breath on exertion and orthopnea. Most individuals also showed decreasing cough effectiveness and decreasing vocal projection. Symptoms were typically slowly progressive and time to diagnosis of ALS was 15 ± 9 months. At the time of diagnosis, most individuals had mild to moderate proximal and distal arm weakness. Bulbar symptoms and leg weakness were less common. Only three of the patients presented with severe respiratory failure, requiring emergency invasive ventilation. One of these three patients was extubated and maintained on BiPAP and the others required long-term invasive ventilation. Twelve patients underwent non-invasive ventilation trials; two patients did not tolerate BiPAP and 10 patients were maintained on long-term BiPAP. The mean survival of the total group from symptom onset to death was 29 ± 20 months and mean survival from symptom onset to death or permanent ventilation was 26 ± 14 months. Mean survival after diagnosis to death or permanent ventilation was 10 ± 9 months.

Conclusions: This study suggests that ALS with respiratory onset is more common in males and that acute presentation requiring intubation is a rare occurrence. Respiratory symptoms tend to be slowly progressive, and ALS with respiratory onset does not typically follow a rapidly progressive course.

Acknowledgement: This study was supported by the MDA.

C91 RESPIRATORY RATE IS STRONGLY PREDICTIVE OF HYPERCAPNIA AND SHORTER SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: There is no consensus on the best marker of respiratory failure in amyotrophic lateral sclerosis (ALS) patients.

Aim: To verify if respiratory rate (RR) is a reliable marker of respiratory impairment in ALS.

Methods: A total of 127 ALS patients (77 males, 50 females; mean age 65 ± 14; 47 bulbar onset and 80 spinal onset) followed from 1996 to 2004 were considered. In order to analyse the effect of respiratory variables, the following parameters were considered: anthropometrics data, type of ALS onset (bulbar vs. spinal), and spirometry parameters at first examination. Non-invasive ventilation was performed in 90 patients, 12 underwent tracheostomy. Survival analysis with exponential distribution was performed. Specificity and sensitivity were calculated.

Results: The median survival time from spirometry was 30 months (95% CI 23–38). Forty-two patients had a RR <20 breaths/min (low RR group) and 85 had a RR $\geqslant 20$ (high RR group). Survival regression with exponential distribution showed a significantly lower median survival time ($p<0.01$) in high RR group (19 months (12–28)) than in low RR group (35 months (26–47)). In the high RR group, VC (63 ± 26% predicted (% pred) vs. 75 ± 20% pred, $p<0.01$), PaO_2 (76 ± 11 mmHg vs. 81 ± 9; $p<0.04$) and MIP (32 ± 20 mmHg vs. 47 ± 26, $p<0.01$) were significantly lower, and $PaCO_2$ (44 ± 9 mmHg vs. 41 ± 5; $p<0.02$) significantly higher. An RR >25 breaths/min (selected from linear regression RR $PaCO_2$) showed 46% sensitivity and 82% specificity in predicting hypercapnia. When spirometric parameters were considered, the best survival predictor was VC (death hazard ratio of 3.56 when VC <50% pred and 1.68 for VC 50–76% pred vs. VC >76% pred.).

Conclusions: In ALS patients a high RR ($\geqslant 20$) can suggest a more severe respiratory involvement and hypercapnia and is related to a shorter median survival time, independently from treatment, and other spirometric or anthropometrics data.

C92 A RANDOMIZED, CONTROLLED TRIAL OF HIGH FREQUENCY CHEST WALL OSCILLATION IN ALS

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease causing progressive weakness and wasting in muscles of the arms, legs, oropharynx and respiratory system. Respiratory muscle weakness is the most common cause of death. Respiratory symptoms include morning headache, breathlessness, fatigue, and sleep disturbance.

Objectives: This study was designed to evaluate the effects of high frequency chest wall oscillation (HFCWO) in ALS patients with respiratory symptoms.

Methods: This was a 12-week randomized, controlled trial of HFCWO in patients with El Escorial defined probable or definite ALS, an ALS Functional Rating Scale respiratory subscale (ALSFRS-RS) $\leqslant 11$ & >5, and FVC$\geqslant$40% predicted. Symptoms, pulmonary function tests (FVC, capnography, O_2 sat, peak expiratory flow (PEF)), level of respiratory support (NPPV, suctioning), ER visits and antibiotic use, ALSA-Q5, Fatigue Severity Scale, ALSFRS-R and measures of dyspnea (Baseline

Dyspnea Index (BDI) and Transitional Dyspnea Index (TDI)) were measured at baseline, four weeks and 12 weeks after entry.

Results: Forty-six patients entered the study (mean age 58.0 ± 9.8 years; 21 males, 25 females); 24 randomized to use HFCWO; 22 to control. Thirty-five completed the trial. Nineteen were using HFCWO; 16 were not. Intent to treat analysis methods were used. There was no significant difference in education, racial/ethnic background, marital status, and employment between treatment groups. Compared to non-users, HFCWO users had significantly less breathlessness ($p=0.021$) and coughed more at night ($p=0.048$) at 12 weeks compared to baseline. After 12 weeks, HCFWO users reported a significant decline in breathlessness ($p=0.048$) compared to non-users; non-users reported significantly more noise when breathing ($p=0.027$). While mean change in PEF increased at 12 weeks compared to baseline for HFCWO users and decreased for non-users, this difference was not statistically significant ($p=0.179$). There was no change in FVC, capnography, or oxygen saturation. More patients in the HFCWO group reported they were easily fatigued ($p=0.046$) at baseline and had severe to moderate functional impairment because of shortness of breath compared to non-users (50 vs. 28.6%; $p=0.170$). No difference was seen between the groups relative to the shortness of breath experienced in relation to the magnitude of the task or the magnitude of the effort. TDI measures change in shortness of breath related to task. More patients using HFCWO reported improvement or no change in magnitude of effort producing shortness of breath (72.2% vs. 43.8%; $p=0.092$) during the 12-week study. There were no differences in the ALSFRS-R and ALSA-Q5 measures with respect to HFCWO use.

Conclusion: Though this was a relatively small trial without a placebo group, subjects assigned to HFCWO had decreased breathlessness and noisy breathing, and increased cough suggesting secretions were mobilized. A larger study of longer duration is needed to determine if HFCWO affects objective measures of disease in ALS.

C93 A RETROSPECTIVE EVALUATION OF HIGH FREQUENCY CHEST WALL OSCILLATION (HFCWO) THERAPY IN PATIENTS WITH ALS/MND

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Introduction: While non-invasive positive pressure ventilation (NPPV) has been shown to extend survival and improve quality of life, there has been a paucity of published data regarding the utility of devices designed to augment expiratory muscle strength and improve airway secretion management in ALS patients.

Objectives: To evaluate the clinical effectiveness of employing a HFCWO device (The SmartVest™ system) in an ALS population.

Methods: A retrospective chart review was performed of 18 ALS patients (10 males, 8 females; mean age 59 years) who had received treatment with The SmartVest™ during the study period from August 2004 to April 2005. Patients were prescribed treatment with The SmartVest™ when the peak cough expiratory flow dropped below 160 l/min. Patients were instructed to use the device twice a day for 10 min/session. Patients were excluded from analysis if they were concomitantly using another cough assist device, had a tracheostomy, or had clinical signs of congestive heart failure or other serious medical comorbidity. The following data were obtained on each patient immediately prior to initiation of The SmartVest™ and again at three months following treatment: ALSFRS-R, forced vital capacity (FVC), maximal inspiratory pressure (MIP), peak cough expiratory flow (PCEF), and daytime oximetry. In addition, following initiation of treatment, patients were asked to complete a satisfaction questionnaire.

Before and after measurements were compared using paired *t*-test. Differences (after minus before) were tested using ANOVA to determine whether the magnitude of the difference was affected by age, gender, bulbar vs. limb disease, length of time between symptom onset and diagnosis of ALS and initial (i.e. at time of enrollment to use of vest) values of endpoints.

Results: Daytime oximetry increased for 8 of 12 patients and the average increase was statistically significant (average increase=1.0 (SE=0.53), one-sided $p=0.04$, $n=12$ patients). None of the other measures (FVC, MIP, PCEF, or ALSFR-S) changed significantly over the three months, although FVC increased slightly (before average=63.5 (SE=6.1) after average=64.6 (5.5), $p=0.29$, $n=14$ patients). A patient survey (completed by 13 patients) revealed that 92% agreed that they felt better after therapy, that their breathing was easier and that insurance should cover the costs. Eighty-five percent agreed that it was easier to clear airways, that the therapy was helping, that it was comfortable and that it had improved their quality of life.

Conclusions: HFCWO is an effective tool for airway secretion management in ALS patients and may also have an impact in improving daytime oxygen saturation. Further studies are necessary to determine the ideal time in which to initiate this intervention and its impact on the incidence of pulmonary infections and hospitalizations.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 64

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SESSION 11 JOINT CLOSING SESSION

C94 INTERNATIONAL ALLIANCE OF ALS/MND ASSOCIATIONS

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The International Alliance of ALS/MND Associations was founded in November 1992 to provide a forum for support and the exchange of information between the worldwide associations. More than 50 national patient support and advocacy groups from over 40 countries worldwide have joined together to form the International Alliance.

The Alliance's objectives are to: 1) increase awareness of ALS/MND worldwide; 2) exchange and disseminate information; 3) improve the quality of care; 4) stimulate and support research; and 5) provide an international identity

It addresses these objectives by delivering the following activities:

- The website – www.alsmndalliance.org
- The International March of ALS/MND Faces banner
- The Alliance Resource Library - www.mndalliancer-esources.org
- The Partnership Program linking organisations in four regions
- The Directory of Associations to facilitate access
- Two awards to recognize significant contributions to people living with ALS/MND. The Forbes Norris Award and the Humanitarian Award.
- Policy documents to assist members, including the Baseline of Services for People Living with ALS/MND, Statement of Good Practice in Drug Trials, and Guidelines for Predictive Testing
- A member hosts the International Symposium on ALS/MND
- Grants to assist members of the Alliance - the Support Grant and the Travel Grant

Our aspirations are to:

- Help member organizations enhance their response to the needs created by ALS/MND including strategies for care, fundraising, and awareness
- Promote access for people with ALS/MND to the best available support no matter where in the world they are
- Be the trusted source of information for members and patients
- Coordinate advocacy with international organizations including drug companies, NGOs and international medical and health organizations
- Maintain the human face of ALS/MND

C95 FROM MIND TO MOVEMENT: NEUROTECHNOLOGIES TO RECONNECT THE BRAIN TO THE WORLD

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Background: Motor neuron disorders such as ALS, as well as spinal cord injury and other paralyzing conditions, prevent movement intentions from being realized. Neurotechnology promises to provide a physical means to restore a new communication link out of the brain when it cannot directly control the muscles. A neuromotor prosthesis (NMP) is a neurotechnology that can detect neural signals reflecting movement intent and convert them into a command. This signal could be used to operate a range of devices, including environmental controls and computer software, in order to restore independence and environmental control to individuals with movement impairments. Devices that use generalized EEG signals as well as the detailed neuronal activity are being developed to provide a physical pathway from the brain to the outside world.

Objectives: This presentation will describe recent developments in NMPs, emphasizing recent results from the Braingate clinical trial.

Methods: The Braingate (Cyberkinetics, Inc) device is approved by the FDA for a 5-patient pilot clinical trial. Two tetraplegic humans have been implanted with a baby aspirin-sized sensor on the cortical surface that records neural activity from multiple neurons in the motor cortex. The sensor is connected to external signal processors and computers that decode electrical signals into a command. The trial is examining safety of the implant and control capabilities.

Results: It is possible to record neural activity in the motor cortex in a person with paralysis and this activity can be modulated by thought alone. No adverse events occurred in the first year. The first Braingate patient has been able to use computer software to open e-mail, for environmental control (TV remote), and to operate robotic hands and limbs.

Discussion: Although at early stages, devices that allow paralyzed humans to use neural activity to operate a range of devices is becoming feasible. Systems will require further development to make them operate without technical oversight. In addition, the devices must be further developed to provide reliable and useful actions for people with paralysis.

Conclusions: Early stage developments in brain interfaces suggest that neurotechnologies are beginning to emerge that may significantly modify the lives of individuals with paralysis from neurodegenerative diseases or CNS trauma.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045267

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Poster Communications

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 67–74

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THEME 1 HUMAN CELL BIOLOGY AND PATHOLOGY

P1 VASCULAR ENDOTHELIAL GROWTH FACTOR IS INCREASED IN THE SKIN OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: AN IMMUNOHISTOCHEMICAL STUDY

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Background: Several studies of skin from patients with amyotrophic lateral sclerosis (ALS) have shown unique morphological and biochemical alterations. Vascular endothelial growth factor (VEGF) is expressed in many tissues and is rapidly up-regulated during hypoxia. It has been shown recently that deletion of the hypoxia response element in the VEGF promoter causes motor neuron degeneration in mice with neuropathological features reminiscent of ALS in humans. It was not clear, however, whether VEGF is involved in the pathogenesis of human ALS.

Objectives: We have carried out an immunohistochemical study of VEGF in skin from ALS patients.

Methods: Skin biopsy samples were taken from the upper left arm of 11 patients with ALS (eight males and three females, mean age 62.1 years) and from 11 controls with other neurodegenerative diseases matched for sex and age (eight males and three females, mean age 59.7 years). Routine formalin-fixed paraffin-embedded 6 μm sections were immunostained according to standard techniques with anti-VEGF antibody. After washing in phosphate-buffered saline, biotinylated anti-IgG was applied. The sections were stained by ABC kit. The immunoreactivity was quantified with an image-analysis system. Statistical comparisons were made by the two-tailed Student's t-test with $p<0.05$ as the significance level. Correlation coefficients were calculated by the least-squares method. Results are expressed as the mean $\pm$ SD.

Results: VEGF immunoreactivity was strongly positive in the epidermis and in some blood vessels and glands of the reticular dermis in all ALS patients. These findings became more conspicuous with ALS progression. Its optical density in ALS patients (6.22 ± 2.91) was significantly higher than in controls (1.65 ± 0.61, $p<0.001$). Furthermore, there was a significantly positive relationship ($r=0.84$, $p<0.001$) between the immunoreactivity and duration of illness in ALS patients. There was no relation between the optical density and the presence of dysphagia, weight loss, muscular atrophy, loss of active movement, and bedridden state in ALS patients or controls.

Conclusions: VEGF was found to be significantly up-regulated in skin samples from ALS patients. The results suggest that changes of VEGF in skin of ALS patients are likely to be related to the disease process.

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510044998

P2 INFLAMMATORY CHEMOKINE RESPONSE DUE TO HYPOXIA IN ALS

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Background: Amyotrophic lateral sclerosis (ALS) is characterized by motor neuron degeneration. Abnormal levels of IL6, TNFα and PGE2 have been demonstrated in ALS and are thought to be related to inflammatory processes. An increase in IL6 has also been shown in obstructive sleep apnoea.

Objectives: To compare IL6 and TNFα levels in CSF and sera of patients with ALS with those of neurological controls, with and without hypoxemia.

Methods: Sera and CSF from 20 ALS sporadic patients (including 10 hypoxemic patients) and 20 non-inflammatory neurological controls (including 10 hypoxemic patients) were analysed. We measured IL6 and TNFα levels using quantitative chemiluminescent assays on the same day with the same kit.

Results: IL6 levels were significantly higher in the CSF and sera of hypoxemic ALS patients compared to the normoxemic ones and were significantly higher in the hypoxemic neurological controls compared to the normoxemic ones. No difference was observed between ALS patients and neurological controls. The same significant results were observed concerning the level of TNFα in sera. TNFα remained undetectable in CSF.

Conclusions: The inflammatory chemokine response in ALS could be related to a normal cellular hypoxia response. The numerous abnormalities described in ALS have to be considered in the light of hypoxemia. Our results suggest a normal response to hypoxemia via NF kappaB and AP-1 in the majority of ALS patients. The impaired response to hypoxemia could involve VEGF via HIF-1.

P3 LACK OF VEGF UP-REGULATION IN CSF FROM ALS HYPOXEMIC PATIENTS

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Background: Deletion of the hypoxia-response element in the VEGF promoter induces motor neuron degeneration in a mouse model. We demonstrated abnormally low levels of VEGF in CSF in early human amyotrophic lateral sclerosis (ALS). VEGF gene expression is mainly stimulated by hypoxia through binding of hypoxia-inducible factor to a defined hypoxia-response element in the gene promoter. Restrictive respiratory deficiency begins early in ALS.

Objectives: To compare CSF and sera VEGF levels, according to hypoxemia, of patients with ALS with those of neurological controls.

Methods: Sera and CSF from 20 ALS sporadic patients, including 10 hypoxemic patients and 20 non-inflammatory neurological controls, including 10 hypoxemic patients, were analysed. We measured VEGF levels using quantitative chemiluminescent assays on the same day with the same kit.

Results: VEGF levels were significantly lower in CSF of ALS patients compared to neurological controls. VEGF levels were significantly higher in CSF and sera of hypoxemic neurological controls compared to the normoxemic ones. No difference was observed between normoxemic and hypoxemic ALS patients. There was a correlation between the severity of hypoxemia and the decreased levels of VEGF.

Conclusion: Compared to hypoxemic neurological controls, we demonstrated a lack of VEGF up-regulation in the CSF of hypoxemic ALS patients. Our results corroborate the involvement of decreased $VEGF_{165}$-dependent neuroprotection during hypoxia in ALS. This paradoxal finding suggests the involvement of different mechanisms impairing the up-regulation of VEGF during hypoxia. Non-invasive ventilation should be administrated earlier to improve survival.

P4 EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS RECEPTORS IN THE CENTRAL NERVOUS SYSTEM IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Vascular endothelial growth factor (VEGF) is a neurotrophic factor which delays disease onset and prolongs survival in the SOD1 mouse model of amyotrophic lateral sclerosis (ALS) (1,2). Dysregulation of VEGF through deletion of the hypoxia response element in its promoter region in mice causes adult-onset motor neuron degeneration that resembles ALS (3). The relevance of these findings in animal models to the human disease is uncertain.

Objectives: To determine the patterns of expression of VEGF and its receptors in the normal human central nervous system, and whether their expression is altered in ALS patients.

Methods: We investigated the expression of VEGF and its major receptors VEGFR2 and neuropilin (NRP1) in the frontal cortex, cervical and lumbar spinal cord of 10 patients with ALS, and matched non-neurological controls, by immunohistochemistry, Western blotting, and quantitative RT-PCR.

Results: Immunohistochemistry demonstrated similar expression patterns of VEGF and VEGFR2 in cervical and lumbar cords, with a finely punctate staining pattern in the neuropil of posterior and anterior horns. Strong expression of both VEGF and VEGFR2 was seen in the somata of anterior horn cells (AHCs), with extension into neurites. In some cases granular staining was seen on the surface of AHCs and major neurites, similar to the pattern obtained for synaptic markers SNAP25 and synaptophysin, raising the possibility of synaptic bouton labelling. A greater proportion of AHCs in ALS cases showed low expression of VEGF ($p=0.006$) and VEGFR2 ($p=0.009$). Immunostaining for VEGF and VEGFR2 was weaker in frontal cortex than in spinal cord, with highest expression in pyramidal neurons. Normal glia did not express VEGF or VEGFR2, but expression of both proteins was seen in reactive astrocytes. Strong expression of NRP1 was seen in the leptomeningeal blood vessels, with weak staining in some AHCs and neuropil. NRP1 expression was seen in reactive glial cells, which occurred more frequently in ALS cases than in controls. NRP1 staining in frontal cortex was generally weaker than in spinal cord. Expression of VEGF, VEGFR2 and NRP1 by motor neurons was confirmed at mRNA level using quantitative RT-PCR, with amplified RNA extracted from laser captured motor neurons from the anterior spinal horn. VEGF expression in punched ventral horn was confirmed by Western blotting.

Conclusion: The similar expression pattern of VEGF and VEGFR2 suggests that this neurotrophic factor may have autocrine/paracrine effects on motor neurons in the spinal cord. A reduction in expression of VEGF and VEGFR2 was seen in AHCs in ALS cases, which would support the hypothesis that, as in mouse models of the disease, reduced VEGF signalling may play a role in the pathogenesis of ALS. NRP1, in comparison, showed a very distinct expression pattern.

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P5 PROTEOLYTIC ACTIVITY IN CEREBROSPINAL FLUID IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by degeneration and loss of motor neurons. ALS arises sporadically in approximately 90% of cases. The mechanisms of motor neuron death in sporadic ALS (SALS) remain unknown. Programmed cell death is considered to be a mechanism of motor neuron degeneration in ALS. Activation of different proteases or dysregulation of proteolytic cascades or the ubiquitin proteasomal complex may be important in development of programmed cell death in ALS.

Objective: The main aim of our study was to research the activity of different proteases in cerebrospinal fluid (CSF) in SALS patients.

Methods: Thirty-eight SALS patients (15 males, 23 females, median age 51.5 years) and 40 controls were included in our study. The average duration of SALS was 19.4 months. All patients were divided into subgroups according to onset of disease, degree of neurological deficit and rate of progression. We measured lactate dehydrogenase (LDH) activity and calpain-like activity as described (1,2) with some pH modifications (pH 7.4 and pH 5.5) in CSF of SALS patients.

Results: LDH activity was significantly increased in the CSF of SALS patients (51.0 ± 24.2 nmol/min/mg protein, $p < 0.05$). Calpain-like activity was increased in CSF in SALS at pH 5.5 ($p < 0.05$) compared to controls but no difference was observed at pH 7.4. Significant correlations were revealed between LDH activity and calpain-like activity in CSF in SALS, but not in control groups. Significant correlations were revealed between enzyme activity and the rate of disease progression, onset of disease and its duration.

Conclusion: The results obtained confirm our proposal about neuronal origin of this proteolytic activity and an important role of proteases in the initiation of motor neuron death.

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P6 MUTANT SOD1 IN CNS AREAS AND PERIPHERAL ORGANS FROM AN ALS PATIENT EXPRESSING G127insTGGG MUTANT SOD1

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Background: More than 110 different mutations in the SOD1 gene have now been associated with ALS. The toxic nature of mutant SOD1s and the reason for the selective vulnerability of motor areas are still unknown. We have previously reported minute amounts of mutant SOD1 in CNS tissue of an ALS patient expressing truncated G127insTGGG (G127X) mutant SOD1 (1). G127X SOD1 was selectively accumulated in ventral horns compared to other parts of the CNS and the protein was highly prone to aggregate. The occurrence and molecular forms of G127X and other mutant SOD1s in peripheral organs has not previously been reported. We here studied tissues from a complete autopsy of a second G127X patient.

Objectives: The aim of this study was to quantify the amount of G127X SOD1 in both non-CNS tissues and in tissues from different parts of the CNS. The biochemical properties of the mutant protein in the different tissues were also investigated. Finally, tissue sections of both CNS and non-CNS tissue were analysed with respect to G127X and wild-type SOD1 by immunohistochemistry.

Methods: An antibody specific for the neo-epitope of G127X SOD1 (1) was used to detect the mutant SOD1 protein on Western blots and tissue sections. As a control,

an antibody raised against the C-terminal end of SOD1 that only recognizes wild-type SOD1 was used. Aggregation of SOD1 was evaluated using centrifugation and filter trap assays as previously described.

Results: In liver and kidney (organs that express the highest levels of wild-type SOD1) the amounts of G127X mutant SOD1 were low, reaching only 0.1% of the normal SOD1 content. Heart and skeletal muscle contained 0.2% and the temporal lobe had slightly higher levels, 0.3%. Ventral horns contained 8% of the normal SOD1 content which is 15–30 times more than liver and kidney. Almost all of the G127X protein, irrespective of tissue, was found in detergent-resistant aggregates. In sections of non-CNS tissues small amounts of G127X protein, predominantly in inclusions, were found. The amounts were, however, much lower than that found in affected CNS areas.

Conclusions: The findings suggest that the CNS areas primarily affected in ALS have difficulties degrading unfolded and aggregation-prone mutant SOD1 compared to other tissues resulting in an impressive accumulation of mutant SOD1 in these areas. This is in agreement with previous results from transgenic mice expressing mutant SOD1s.

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P7 SUBCELLULAR FUNCTIONAL SPECIFICITY OF DYNEIN-DYNACTIN COMPLEX SUBUNITS: DISTURBANCES IN NEURODEGENERATIVE DISEASE

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Background: Cytoplasmic dynein and its activating complex dynactin drive the retrograde transport of material necessary for the survival and proper functioning of neurons. Disruption of the dynein-dynactin complex by either overexpression of the dynamitin subunit or by mutations within individual dynein/dynactin subunits can cause motor neuron degeneration.

Objectives: To examine the distribution of the dynein complex subunits in the CNS, to test the hypothesis that this may be altered in motor neuron disease (MND) and in the *Loa* mouse in which there is a mutation in the dynein heavy chain.

Methods: Three dynactin subunits (p150, dynamitin p50 and p62) and two subunits of the conventional cytoplasmic dynein (dynein heavy chain and dynein intermediate chain) were studied in the central nervous system of human post-mortem sections from MND, Alzheimer's disease, and patients with no neurological disease. *Loa* heterozygous mice and wild-type controls were also studied. Immunohistochemical studies of formalin fixed paraffin embedded tissue sections were performed, and validated by multiple antibodies and Western blotting.

Results: Unexpectedly, coordinated distribution of dynein-dynactin complex subunits was not evident, even in normal tissues. There was no observable difference in the general cellular and structural localization of complex subunits between *Loa* heterozygotes and wild-type mice, or in MND cases compared to the Alzheimer's disease and control sections. However, the localization of certain complex subunits, but not others, to pathological structures, including inclusions in MND, plaques, granulovacuolar degeneration and neurofibrillary tangles in Alzheimer's disease suggests a role in pathological features of neurodegeneration.

Conclusion: The results suggest that different subunits of the dynein-dynactin complex may have different roles in subcellular function, and that primary events that disturb the function of individual components may have differential primary effects on subcellular structures.

P8 ULTRASTRUCTURAL STUDY OF MITOCHONDRIA IN THE SPINAL CORD OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: Recent studies have found increasing substantial evidence for the involvement of mitochondrial damage in motor neuron degeneration in familial (with SOD1 mutation) and sporadic amyotrophic lateral sclerosis (ALS), and mutant SOD1 transgenic mice. However, little information is available about morphological changes in mitochondria in sporadic ALS.

Objective: To examine morphological changes of mitochondria in the spinal cord of sporadic ALS patients by electron microscopy.

Methods: We studied the spinal cords of 14 patients (ages 49–83, average 68.3 years) with ALS, and 15 age-matched control individuals (ages 44–80, average 62.7 years) with no neurological disease. All post-mortem investigations were performed within 6 h of death. In all cases, tissue blocks were obtained at autopsy from the lumbar spinal cord (L1–L5), and anterior horns of each level were fixed immediately with 2% glutaraldehyde in phosphate buffer (pH 7.4). After ordinary procedures, each embedded tissue block was subsequently cut into semi-thin sections. Appropriate portions of the semi-thin sections were cut into ultra-thin sections, and stained with uranyl acetate and lead citrate for electron microscopy.

Results: Swollen mitochondria with markedly increased cristae were occasionally observed in the somata of anterior horn neurons in one control patient and marked accumulation of mitochondria were seen in the somata in another control patient. Seven ALS patients showed a stack of multilayered cristae consisting of linear structures in the inner compartment of mitochondria. These inclusions were observed in mitochondria mainly of the somata and only occasionally of the axons. Periodic transverse processes like rungs of a ladder were seen in the intracristal space in two ALS patients, in the intermembrane space in two patients, and both in the intracristal and intermembrane space in one patient. They were observed in mitochondria predominantly of the somata and only occasionally of the axons. Three ALS patients had stubby mitochondria having periodic, stubby protrusions on the outer membrane. Two ALS patients exhibited swollen mitochondria with markedly increased cristae in the somata of anterior horn cells. Marked accumulation of mitochondria was found in the somata of four ALS patients, and in the dendrites and proximal axons (axon hillock and initial segment) of two patients, respectively. Electron-dense round inclusion bodies were seen in the inner compartment of mitochondria in almost all of the controls and sporadic ALS patients.

Conclusions: Various kinds of morphological alterations of mitochondria were observed in sporadic ALS, which may be correlated with the pathological mechanism of degenerative processes of anterior horn neurons in this disease.

P9 MITOCHONDRIAL CHANGES IN SKELETAL MUSCLE OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS AND OTHER NEUROGENIC ATROPHIES

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Background: There are conflicting results on specific mitochondrial dysfunction in skeletal muscle of patients with amyotrophic lateral sclerosis (ALS) (1,2). This is at least partly due to the lack of adequate diseased controls with neurogenic atrophies other than ALS.

Objectives: To investigate whether the dysfunction of muscle mitochondria in ALS is specific.

Methods: We investigated the histochemical distribution of mitochondrial marker activities, the ratio of mitochondrial (mt) versus nuclear (n) DNA, and the activities of citrate synthase (CS) and respiratory chain enzymes in muscle biopsies of 24 ALS patients. The data were compared with data from 23 patients with other neurogenic atrophies (NA), and 21 healthy controls.

Results: Muscle histology revealed similar signs of focally diminished mitochondrial oxidation activity in muscle fibres in both diseased groups. There was only a marginal decline of mt/n DNA ratios in ALS and NA in comparison to healthy controls. The specific activities of mitochondrial markers CS and SDH were significantly increased in both ALS and NA patients. The activities of respiratory chain enzymes related per gram wet weight as well as per gram non-collagen protein were not significantly different in all three groups.

Conclusion: The histochemical, biochemical, and molecular mitochondrial changes in muscle are not specific for ALS, but also accompany other NA.

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P10 GROWTH HORMONE SECRETION IS IMPAIRED IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disorder in human adults, characterized by selective and progressive upper and lower motor neuron degeneration in spinal cord, brainstem and motor cortex. Few studies on endocrine abnormalities, including a reduction in serum insulin-like growth factor I (IGF-I) concentration have been reported.

Objectives: To investigate the growth hormone (GH)-IGF-I axis in ALS patients.

Methods: GH-releasing hormone and arginine tests were performed on 39 ALS patients (21 males and 18 females; age range 44–82 years; mean age 61 years): blood samples for GH were collected at baseline, and after 30 and 60 min; IGF-I was determined at baseline only. The control group consisted of 35 age- and sex-matched normal subjects (12 males and 23 females; age range 40–77 years; mean age 50 years). Ten patients were also studied after stopping riluzole therapy for one month.

Results: Mean basal GH levels ($\pm$SD) were significantly reduced in ALS patients compared with normal controls (0.64 ± 1.8 vs. 1.3 ± 0.3 ng/ml; $p < 0.01$), as well as peak GH concentrations (14 ± 10 vs 38.0 ± 3.5 ng/ml; $p = 0.01$). Fourteen patients (35%) showed a normal GH response to stimulus (peak GH$\geqslant$16 ng/ml); 12 patients (30%) displayed a moderate GH deficiency ($9 \leqslant$peak GH<16 ng/ml); in 14 patients (35%) GH response was

markedly deficient (peak GH < 9 ng/ml). IGF-I levels (140 ± 60 ng/ml) were significantly reduced in ALS patients compared to normal subjects (220.4 ± 18.6). No significant correlation was observed between peak GH concentrations and age, BMI, disease duration or drugs.

Conclusion: Our data indicate a reduction of GH secretion in ALS patients: mechanisms behind these findings need to be clarified.

P11 ENDOCRINOLOGICAL DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: The data on endocrinological dysfunction in ALS are heterogenic. Comorbidity to thyroid disorders is proven. More information about the pathophysiological impact of the endocrine system in ALS is required.

Objective: This prospective, controlled study aimed to evaluate the correlation of endocrinological dysfunction and cognitive or affective impairment in ALS.

Methods: Eighteen patients (eight females, 10 males, mean age 59.6 years) with sporadic ALS confirmed by El Escorial criteria were included. Fifteen had generalized ALS with bulbar affection. Three patients were without bulbar symptoms. All patients were treated with riluzole and 12 patients with vitamin E. The control group were 10 patients (seven females, three males, mean age 55.8 years), six with cerebral ischaemia, one with multiple sclerosis, one with epilepsy, one with chronic headache and one with poliomyelitis. The serum blood levels of TSH, fT3, fT4, PTH, LH, FSH, prolactin, oestradiol, calcium and creatinine kinase were determined. The neuropsychological assessment was evaluated by SDMT, Cognitive Failures Questionnaire, HADS, FSS, Digit span test, Pre-Sleep Arousal Scale and SF 36. The statistical analyses were performed with Kruskal Wallis *H*-tests, Mann Whitney *U*-Tests and Fischer-tests.

Results: The only significant difference between ALS and controls was an elevated fT4 level in ALS patients ($p < 0.04$). There was no difference in the TSH levels. Concerning the neuropsychological tests there was no significant difference between ALS patients and controls. In both groups a trend to higher cortisol and ACTH levels in affective impairment (HADS) could be observed.

Conclusion: This study confirms the role of thyroid dysfunction in ALS, suggesting a dysregulation of the hypothalamo-pituitary-thyroid axis in ALS. The higher disease stages in our ALS patients and the influence of riluzole and vitamin E as possible reasons for our different results compared to former studies have to be discussed.

P12 MODIFIED EXPRESSION OF ANTIOXIDANT ENZYMES IN LYMPHOCYTES OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Amyotrophic lateral sclerosis (ALS) is a sporadic neurodegenerative disease for 90% of cases. Almost all experimental approaches are focused on the SOD1 gene, responsible for 20% of familial cases (2% of total patients). Nevertheless sporadic (SALS) and familial (FALS) patients show an overlapping clinical picture. Both FALS and SALS display heightened markers of oxidative injury suggesting a common pathogenic mechanism. In previous works (1,2), it has been demonstrated that lymphocytes of SALS patients show abnormalities of protein expression and calcium and mitochondrial metabolism, suggesting that traits of the disease, as already observed for other neurodegenerative diseases (3) are present also in the peripheral blood cells.

Objectives: The aim of this study was to investigate the expression of two antioxidant enzymes Bcl-2 and SOD1, implicated in FALS, in lymphocytes from SALS patients both in basal conditions and after oxidative stress induced by H_2O_2 treatment.

Methods: Ten sporadic ALS patients, free from pharmacological treatment, and 10 age- and sex-matched healthy volunteers were included in the study. Lymphocytes obtained by Ficoll-Hystopaque 1077 gradient were processed for protein extraction immediately after isolation and after 8 or 18 h incubation with 100 μM H_2O_2. The Bcl-2 and SOD1 expression in basal conditions and after oxidative stress in lymphocytes of controls and SALS was evaluated by Western blotting.

Results: Western blot experiments showed that expression of Bcl-2 and SOD1 are significantly reduced in SALS ($p < 0.001$, Student's *t*-test) (Bcl-2: 1.29 ± 0.13, arbitrary units, Bcl-2/b-actin; SOD1: 0.84 ± 0.06, arbitrary units, SOD1/b-actin) than in control lymphocytes (Bcl-2: 4.25 ± 0.55, arbitrary units, Bcl-2/b-actin; SOD1: 2.35 ± 0.15, arbitrary units, SOD1/b-actin). The 8 and 18 h oxidative stress significantly reduced the amount of Bcl-2 and SOD1 in control lymphocytes ($p < 0.01$, ANOVA and Newman Keuls' *Q*-test) but no variation in Bcl-2 and SOD1 expression was detected in SALS lymphocytes.

Conclusions: In basal conditions, Bcl-2 and SOD1 proteins are differently expressed in SALS, and control

lymphocytes could be strictly related to the enhanced oxidative stress detected in SALS patients. Additional oxidative stress does not modify the expression of Bcl-2 and SOD1 proteins in SALS lymphocytes, even after 18 h stress. These data suggest that in SALS patients the oxidative pathway is deregulated and can not be modulated any more.

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P13 MCP-1 CHEMOKINE RECEPTOR CCR2 IS ABSENT ON CIRCULATING MONOCYTES IN SPORADIC ALS

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Background: Recent studies on blood specimens from patients with sporadic ALS (SALS) found elevated levels of abnormally activated monocyte/macrophages (MO) compared to controls. In an attempt to identify why these MOs were activated we evaluated the relationship between the recently SALS associated MO chemokine, monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR2. Theoretically, MCP-1, a potent chemoattractant and MO activating factor, might be a key trigger for mediating chemotaxis of MOs to areas of neurodegeneration in SALS.

Objective: To determine CCR2 expression on circulating monocytes and plasma MCP-1 levels in SALS patients compared to normal and diseased (age related macular degeneration, ARMD) controls, and to determine if plasma MCP-1 levels in SALS patients and/or CCR2 expression on monocytes correlate with clinical stage of disease.

Methods: Flow cytometry and ELISA quantitation were performed to determine CCR2 expression median fluorescence intensity (MFI) on blood monocytes and levels of plasma MCP-1 in heparinized blood samples from 21 patients with SALS, 25 age-matched normal control subjects, and 25 ARMD patients. Results from immune studies were evaluated in relation to the severity of neurological impairment as determined by the revised ALS Functional Rating Scale (ALSFRS-R).

Results: Compared to the control population (266 ± 87 pg/ml), significantly higher levels of plasma MCP-1 were shown in SALS patients (373 ± 102 pg/ml, $p < 0.001$) and ARMD (364 ± 167 pg/ml, $p < 0.01$). Plasma MCP-1 levels between two disease groups of SALS and ARMD were similar. The MCP-1 receptor expression (CCR2 MFI) was dramatically lower on SALS CD14 monocytes (MFI CD14+CCR2+$= 6.2 \pm 8.4$, $p < 0.001$) compared to control subjects (MFI CD14+CCR2+$= 33.4 \pm 22.2$). In contrast, the disease control ARMD specimens with high plasma levels of MCP-1 showed normal levels of CCR2 expression (MFI 14+CCR2+$= 23.5 \pm 15.6$). There was no correlation between plasma MCP-1 levels, or degree of MO CCR2 expression and clinical stage of disease.

Conclusion: The current study is the first to identify the loss of a critical MO chemokine receptor, CCR2, in patients with SALS. Considering that another chronic MO activation disease, ARMD, had similar elevated levels of MCP-1, it is unlikely that this loss is through a receptor-ligand mediated down-regulation. Rather, it suggests that the SALS MO differentiation state may be somehow unique and potentially contribute to the pathogenic complications that occur in patients with SALS. Conceivably, this loss of CCR2 may contribute to the development of a disease specific biomarker of SALS.

P14 TRANSCRIPTIONAL PROGRAM OF PERIPHERAL BLOOD CELLS IN ALS: EVIDENCE FOR SYSTEMIC IMMUNE ACTIVATION

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Background: Individuals with amyotrophic lateral sclerosis (ALS) have elevated levels of activated macrophages in their blood, suggesting that systemic immune activation is one hallmark of disease. One path toward obtaining greater understanding of the inflammatory processes associated with ALS is through the use of microarray technologies for quantitating gene transcription. Previously, microarray analysis has been performed on post-mortem spinal cords, but not in other tissues from ALS patients.

Objective: To use microarray technology to determine the overall transcriptional program of peripheral blood cells in ALS patients and controls.

Methods: Blood was collected from 22 patients with ALS (14 males, 8 females, average age 61 years) and 20 controls (7 males, 13 females, average age 61 years) after obtaining informed consent. Peripheral blood mononuclear cells were obtained by Percoll gradient centrifugation and cultured overnight at 37°C under non-adherent conditions

to allow recovery from the isolation procedure. Cells were then collected, lysed, and total RNA prepared. The quantity and integrity of the total RNA was verified using an Agilent 2100 Bioanalyzer. RNA samples of acceptable quality were then prepared, hybridized to Affymetrix HGU133plus 2.0 chips, and scanned using Affymetrix equipment and protocols. Probe signals were calculated using GCOS software. Array results were analyzed using Excel, GenMAPP, and GeneSpring software packages; Gene Ontology (GO) analysis was performed with MAPPFinder software and the significance of associations rated by Z score and permuted p value with multiple testing correction.

Results: Approximately 40% of the probes had signals of 50 or greater (max signal > 22,000) in eight or more of the ALS or six or more of the control samples. Of those probes ~7% were up-regulated 2-fold or greater and 2% were down-regulated 2-fold or greater in ALS patients relative to healthy controls. GO analysis did not find evidence that any class of gene was down-regulated significantly more often than expected by chance. In contrast, GO analysis demonstrated significant up-regulation of genes associated with the immune or inflammatory response including genes associated with the GO term 'Response to Biotic Stimulus' (z score = 15.16, $p < 0.001$) or the 'Immune Response' (z score = 15.55, $p < 0.001$). Up-regulated genes include those involved in apoptosis, signal transduction, chemokine dependent signalling, neutrophil mobilization, and cell adhesion.

Conclusions: Microarray analyses of peripheral blood cells confirm systemic immune activation in ALS. Genes identified by this analysis have potential utility as novel biomarkers or therapeutic targets.

P15 ANALYSIS OF HEMATOPOIETIC PROGENITOR CELLS IN ALS PATIENTS

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Background: Amyotrophic lateral sclerosis (ALS) is a lethal disease characterized by the death of motor neurons (MNs) where many variables remain unclear, making it difficult to assess an effective treatment. Autologous stem cell (SC) therapy may be a promising strategy for restoring lost neuro-motor function or preventing degeneration of MNs. A preliminary clinical trial using bone marrow (BM) SC transplantation is currently in progress; however, patient normal hematopoietic functionality has still to be proven.

Objectives: In order to assess BM normal hematopoietic potential, we analysed the *in vitro* characteristics of BM hematopoietic and stromal progenitors in 21 sporadic patients and one familia1 ALS patient (A4V SOD1).

Methods: To evaluate the mesenchymal compartment, both fibroblast colony-forming cells or CFU-F (described as rapidly adherent clonogenic cells in which progeny is designated as marrow stromal fibroblasts) and mesenchymal cell cultures obtained by plastic adherence were prepared. To investigate both the functional properties of hematopoietic primitive and the supportive capacities of their stromal cells, long term cultures were accomplished and supernatants of the cultures seeded in semi-solid methylcellulose supplemented with specific hematopoietic growth factors. The number of myeloid colonies was defined.

Results: Preliminary results showed a defective mesenchymal compartment. Reduced growth of mesenchymal stem cells was observed and cultures were shown to be contaminated by hematopoietic cells but normal functions and growth were recovered after trypsinization. Moreover, the number of colonies obtained by CFU-F assay was reduced (median number of colonies: two in ALS patients versus 16 in normal controls). No significant differences were observed between the hematopoietic compartment of normal and ALS patients. The ALS patients' stromal cells grow slightly faster than normal controls; an increase in the number of colonies was observed starting from the first week of culture in ALS patients, confirming the faster stromal growth.

Conclusion: These preliminary data suggest that in addition to a defective mesenchymal compartment, delayed activation of mesenchymal precursors may be present in sporadic and familial ALS patients. Further investigations are needed to fully explain the biological significance of these results and to better address the SC therapeutic approach.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 75–90

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THEME 2 GENETICS AND EPIDEMIOLOGY

P16 THE SOD1 TRANSGENE IN THE G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS LIES ON DISTAL MOUSE CHROMOSOME 12

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Background: The SOD1 G93A transgenic mouse strain is a widely studied model of amyotrophic lateral sclerosis (1). These mice carry a human mutant Cu/Zn superoxide dismutase transgene array and have been used in many breeding experiments to look for interactions with other loci, including transgenic and gene targeted mutations. The transgene insertion site is pivotal as this may affect the outcome of such breeding experiments.

Objective: To map the SOD1 transgene in the G93A mouse model of amyotrophic lateral sclerosis by fluorescence *in situ* hybridization (FISH).

Methods: Metaphase spreads were made from SOD1 G93A bone marrow. Plasmid pHG-SOD1^{WT}, containing a human wild-type SOD1 genomic insert (2), was used as a probe to detect the human transgene array in a FISH experiment.

Results: By analysing more than 20 metaphase spreads in the FISH mapping experiment, we determined that the SOD1 G93A transgene insertion site lies on distal mouse chromosome 12. The 'Legs at odd angles' (*Loa*) locus, which is an entirely unrelated mutation in the dynein 1 heavy chain 1 gene (*Dync1h1*), also maps to this chromosome (3). Analysis of a SOD1 G93A x *Loa* cross determined that the site of the transgene insertion lies proximal of the *Dync1h1* gene, on mouse chromosome 12.

Conclusions: We have mapped the SOD1 G93A transgene array to mouse chromosome 12 in band E, by FISH. By analysing a small data set from an existing cross we find the *Loa* mutation in the *Dync1h1* gene lies roughly 31cM distal in what is thought to be band F2. The exact correlation between bands and genetic and physical distances remains to be determined, but it is clear that the two loci are linked, although a considerable distance apart.

The transgene mapping data are important for our SOD1 G93A x *Loa* cross, as knowledge that the two loci are linked now explains the low number of wild-type mice seen in our *Loa* SOD1 x *Loa*+ cross. The position of the transgene array is also relevant to other laboratories carrying out crosses with genes/transgenes of interest on mouse chromosome 12. We believe it would be of interest to the ALS community to determine the site of transgene insertion for other SOD1 mutations.

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P17 UP-REGULATION OF GENES IN MURINE SPINAL CORD TRANSCRIPTOME

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Background: Analysis of the transcriptome in motor neurons, the cells traditionally associated with amyotrophic lateral sclerosis, is hampered both by the diversity of the cell types in nervous tissue as well as the cloaking of the neural elements by the overwhelming glial matrix surrounding these cells. Using a combination of suppression subtractive hybridization (SSH), mirror orientation subtraction (MOS) and normalization of cDNA libraries from spinal cord materials with common glial transcriptome elements, we have constructed a modified library of differentially expressed genes that appear to be up-regulated in spinal cord.

Methods: A differentially expressed cDNA library from murine tissue was constructed using a combination of SSH, MOS and normalization against common glial cDNA sequences. Differential screening using dot blot arrays of clones from these libraries were probed with cDNA derived from forward and reverse subtracted spinal cord and visual cortical cDNA. Positive clones from these screened libraries are confirmed with RT-PCR using Islet-1 (a motor neuron marker) and neuron specific enolase as positive controls.

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045005

Results: One hundred and sixty clones, 20% of 800 randomly selected clones derived from the forward subtracted libraries, were up-regulated in spinal cord tissue when cDNA from spinal cord was used as the probe. Using RT-PCR, we have confirmed five clones that are moderately differentially expressed (1 to 1.5-fold increased), one clone that is differentially expressed (1.5 to 2-fold increased) and one clone that is strongly differentially expressed (greater than 2-fold increased).

Discussion: Sequencing analysis of the cDNA derived from these clones shows a mixture of genes with known and unknown function expressed in the spinal cord compared with visual cerebral cortex. Final confirmation of differential expression by *in situ* and northern blot hybridization and analysis of the possible roles of the proteins produced from these genes and their significance in motor neuron function is ongoing.

P18 A GENOME-WIDE ASSOCIATION STUDY IN SPORADIC ALS USING DNA MICROARRAYS

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Background: Most cases of ALS occur sporadically, however twin studies suggest that the genetic contribution to disease development may be up to 85%. Previous association studies have targeted candidate genes but have yielded few positive, reproducible associations. We have previously used DNA pooling in a whole-genome association study using microsatellites, but it is time consuming to genotype microsatellites in very large numbers using current technology. DNA microarrays allow the mass genotyping of much larger numbers of SNPs than is feasible with microsatellites, and combining this approach with DNA pooling provides a quick and efficient method of performing a genome-wide screen.

Objectives: To perform a genome-wide screen for susceptibility and phenotype modifier genes for sporadic amyotrophic lateral sclerosis (ALS), using DNA pools genotyped on Affymetrix GeneChip© microarrays.

Methods: Cases were selected and matched for sex and age within one year. Pools were genotyped on the Affymetrix GeneChip© 10K Mapping microarrays. Individuals were also genotyped on the microarrays to produce correction factors which adjust the data for differential hybridization.

Results: Pools were constructed of 300 cases and 300 matching controls. Thirty-three individuals were genotyped for data correction. Each pool was hybridized to the microarray in triplicate and median values used for analysis. 11473 SNPs were analysable. Correction factors were available for 9990 SNPs. Using a modified χ^2 which takes measurement error into account, 582 markers were significant at $p<0.05$, and 39 were significant at $p<10^{-7}$. The largest effect sizes were seen for SNPs on chromosome 7 (OR = 15.9, $p<10^{-11}$) and chromosome 3 (OR = 12.3, $p<10^{-18}$).

Discussion and conclusions: DNA microarrays provide the high-throughput technology which genome-wide association studies require. This initial work shows that genotyping DNA pools on microarrays is a useful method for first-pass screening which has quickly and efficiently identified regions associated with ALS to target with fine-mapping studies.

P19 HAPLOTYPE STUDIES IN SMALL ALS FAMILIES: USEFULNESS IN THE SEARCH FOR NEW GENES CAUSING ALS

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Background: Familial ALS forms the tip of the iceberg of motor neuron disorders. Families with ALS can be a powerful tool for finding new genes and may provide valuable clues to the biology of motor neuron functioning. In a small ALS family, we have excluded all known causes of autosomal dominant ALS by analysing the reconstructed haplotype and propose that this family may be associated with a new locus for familial ALS.

Objectives:

1. To find new genes which singly or in combination cause ALS.
2. To examine ALS families which do not have SOD1 mutations in order to locate additional motor neuron disease-causing genes.
3. To store and bank DNA samples (and transformed lymphocyte cell lines) from our study families for future research.

Method: We have been recruiting ALS families for genetic studies for the past 12 years. Eighty-six families with ALS lack the SOD1 mutation and show autosomal dominant inheritance. These families are being tested for the known loci by linkage and/or haplotype analysis. DNA was extracted from whole blood using the Puregene DNA isolation kit. Genotyping for ALS loci was carried out by PCR amplification with short tandem repeat markers at these loci. For affected, deceased individuals whose DNA samples are unavailable, we have reconstructed the haplotypes with spouse and children's DNA.

Results: One of our large SOD1 negative ALS families has a theoretical linkage score (Zmax) 2.7 on simulation analyses. The results of pairwise analyses between disease phenotype in our family and the ALS loci yielded

negative 2-point LOD scores at recombination factor 0; however, the results are not significant to exclude these loci. Using manually reconstructed haplotypes in this family we have been able to exclude all four loci for autosomal dominant ALS without associated fronto-temporal dementia.

Conclusion: Genetic analyses in late onset disease conditions with low disease penetrance may not provide conclusive results. This study shows the difficulties associated with such studies, which may be overcome by running more markers or genotyping individuals in the lower generations to build haplotype information for individuals in the older generation. Thus, small families could provide supporting evidence for new loci or to screen for genes that have been found in larger families.

P20 A NATIONAL NETWORK ON FAMILIAL ALS: AN UPDATE ON COLLECTION AND COLLABORATIONS

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Background: Familial ALS (FALS) represents 10 to 20% of ALS cases. However, only a small part is multi-generational. Moreover, in those multigenerational families SOD1 mutations are frequent (50%). This largely limits the potential of linkage studies in ALS. A large recruitment of FALS cases is necessary to allow such studies to be performed.

Objectives: To describe the organisation of a national network on FALS and its ability to identify families for future genetic work, and to present an update of recruitment up to 2004.

Methods: A national network on FALS was created in 2000. The large majority of university centres are involved. DNA is extracted and cell lines are established to allow genetic studies to be performed. Clinical data are prospectively collected: age of onset, site of onset, presence and diffusion of lower and upper motor neuron signs, date of death. The pedigree is established for all FALS case. For each family, SOD1 mutations are searched for at least one member with ALS. When SOD1 mutations are absent, a CRA manages to collect the family.

Results: Since 2000, 150 new families have been identified and the FALS index-case collected. Almost 80% of the families are composed only of 2 ALS cases. The remaining 20% are multigenerational. We identified a total of 18 families with SOD1 mutations: one with recessive D90A, one compound heterozygote and the other with dominant inheritance. In a large family without SOD1 mutations, genome screen allowed to describe a new locus on chromosome 18q. In 2004, 29 new families have been recruited. Four of those families are multigenerational. In two of them, a SOD1 mutation could be found, confirming our previously published results.

Discussion: Multigenerational, dominant FALS cases are rare. The development of a national network is a useful tool to allow new loci to be found. Collaboration with North America (Professor G. Rouleau) is underway for that proposal. Such a network may also allow sib pair studies to be carried out. A specific programme is underway in a large European collaboration on that topic (Dr. C. Shaw, Dr. J. De Belleroche). It remains surprising that such a large number of families are not multi-generational. Are they dominant with low penetrance, recessive or multigenic? A candidate gene approach (Dr. Corcia, Professor Andres) in those cases could give clues to that point.

Acknowledgement: On behalf of the French group on motor neuron ALS research we would like to thank all the physicians who participate in the network. This is an open network; please do not hesitate to contact us with new ideas.

P21 A NOVEL MUTATION (GLU133VAL) IN CU/ZN SUPEROXIDE DISMUTASE CAUSING FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS IN CHINA

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Background and objective: More than 100 mutations in SOD1 gene have been described in ALS (1). Here we present a two-generation family with ALS from Liaoning province, North China, which has a novel mutation in SOD1 gene.

Methods: We studied the family including four ALS patients and 50 unrelated healthy controls. Exons 1–5 of the SOD1 gene were amplified, the fragments were gel purified and sequence analysis performed.

Results: The proband is a member of a family of Chinese descent in which at least four individuals in two successive generations have displayed clinical evidence of ALS. The pattern of inheritance of the disease trait suggests autosomal dominance. A novel mutation (A to T) in exon 5 of the SOD1 gene, which is predicted to result in the replacement of Glu by Val at codon 133 (E133V), was identified in the proband. We found no other abnormality in the PCR products of other exons. To determine whether the E133V mutation could be a polymorphism in a Chinese population or not, 50 control subjects were tested by the same method but no abnormality was found.

Discussion: In the present study, we have shown that a Chinese kindred with ALS is associated with a missense mutation in exon 5 of the SOD1 gene. The region affected by the E133V mutation is VII active site loop, which has

also been affected by more than 10 other mutations (1,2). The clinical phenotype within members of this family is relatively variable.

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P22 SOD1 GENE MUTATIONS IN ITALIAN PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: The SOD1 gene, encoding Cu/Zn superoxide dismutase, is mutated in 20% of familial amyotrophic lateral sclerosis (FALS) and about 5% of sporadic (SALS) cases. More than 100 SOD1 gene mutations have been identified.

Objectives: Analysis of SOD1 mutations in Italian ALS patients.

Methods: Mutations were searched by DHPLC and direct sequencing of the 5 exons, exon/intron boundaries and 3'UTR in 44 patients (29 male and 15 female: mean age 54.3 $\pm$ 14.5 years) consecutively referring to our ALS centre from all Italian regions. Written informed consent was obtained from all patients.

Results: We found SOD1 mutations in 3 out of 41 (7.3%) putative SALS patients and no mutations in the 3 FALS cases. In a 50-year-old female we detected the N65S mutation. Disease onset occurred about 10 years ago with a dropped foot. At the latest visit she showed a prevalent involvement of the lower motor neuron in the lower limb with a mild functional impairment (ALS-FRS = 34). Upper motor neuron involvement was expressed only by an abnormal plantar reflex. A new heterozygous change AAG to TAG, introducing a stop codon at position 136 in exon 5, was found in a 44-year-old male. The onset of the disease was characterized by a rapid progressive wasting of muscular strength with hyperreflexia in the left lower limb one year ago. At the time of our visit the patient showed upper and lower motor neuron involvement only at the spinal level with mild functional impairment (ALS-FRS = 37). The third mutation, A95T in exon 4, was identified in a 64-year-old female . Onset of the disease occurred about 20 years ago with a dropped foot. At the present time she showed a severe tetraparesis with involvement of both types of motor neurons.

Conclusions: Of the three detected mutations, N65S has been described previously in three cases, one of which was familial. They showed a slow progression of the disease with prevalent lower motor neuron involvement as in our case. The patient with the K136X mutation showed a rapid progression of the disease, in agreement with previously reported familial cases carrying truncating mutations in exon 5 (L126X and G141X). The A95T mutation, detected in a very slowly progressive patient, was previously identified in one Italian patient with juvenile onset and slow progression of the disease, but also in his unaffected relatives suggesting low penetrance of the mutation. The similarity of the clinical pictures between previously described FALS and our SALS cases links these mutations to specific clinical forms and strengthens the need to extend genetic analysis also to apparently sporadic cases.

P23 THE 'D90A' STILL AN ENIGMA AMONG SOD1 GENE MUTATIONS: REPORT OF THREE ITALIAN CASES

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Background: Of all the SOD1 gene mutations described, uniquely the D90A mutation has been identified in recessive, dominant and apparently sporadic cases. All the homozygous patients showed a typical uniform diphasic phenotype with an insidious onset. The mean age at onset is 44 years and the median survival time is 14 years. A few sporadic and familial ALS patients heterozygous for the D90A mutation have also been described, characterized by more variable phenotype and course.

Objectives: To describe atypical clinical features of two D90A heterozygous subjects belonging to unrelated families and one apparently sporadic D90A heterozygous ALS patient. Also, to evaluate a possible common haplotype of these subjects.

Methods: Mutation analysis of the SOD1 gene was carried out by single strand conformational polymorphism (SSCP) analysis followed by direct sequence of sample with abnormal migration patterns. A haplotype study was performed using eight polymorphic microsatellite markers flanking the SOD1 locus. Microsatellites were amplified by fluorescent polymerase chain reaction (PCR) primers and analysed with an automated sequencer.

Results:
Case 1. A 42-year-old male showed clinical and electrophysiological findings inconsistent with typical motor neuron disease and suggestive of a multiple sensory-motor peripheral neuropathy. His maternal grandfather died at 63 years of age with ALS. Segregation analysis of the

mutation in the family showed three healthy heterozygous relatives (45, 76 and 78 years old).

Case 2. A female with paternal history of ALS showed a bulbar onset of the disease at age 71 years and died three years later. No SOD1 mutation was found in the patient. Intriguingly, her 65-year-old healthy brother is heterozygous for the mutation.

Case 3. A 58-year-old male, heterozygous for the D90A mutation, presented progressive signs of motor neuron disease in three limbs with onset two years before. His family history was negative.

A common haplotype was found in our pedigrees and in the sporadic patient.

Discussion: These cases provide further evidence that the D90A mutation still has an unclear pathogenetic role in the disease and represents an unresolved challenge among all SOD1 mutations. Indeed, in the first pedigree a dominant inheritance with incomplete penetrance could be hypothesized. Alternatively the patient could suffer from a coincidental undiagnosed disease of peripheral nerves and belong to a recessive pedigree. In the second pedigree the mutation was found in a healthy brother but not in the patient affected by typical ALS. The third patient represents the first Italian apparently sporadic ALS case with heterozygous D90A mutation.

P24 A PHENOTYPIC-GENOTYPIC STUDY OF AMYOTROPHIC LATERAL SCLEROSIS ITALIAN FAMILIES WITH THE G41S SOD1 GENE MUTATION

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Background: More than 100 different mutations in the superoxide dismutase gene (SOD1) have been found worldwide in patients with amyotrophic lateral sclerosis (ALS), some occurring as recurrent mutations or as founder mutations. G41S is the predominant SOD1 gene mutation identified so far in ALS Italian families, together with the L84F. Occasionally, specific mutations are associated with a particular phenotype.

Objectives: To evaluate a possible genotype-phenotype correlation in six patients belonging to four unrelated familial ALS (FALS) pedigrees originating from north-west Tuscany in central Italy, carrying the G41S mutation and to investigate for a founder effect for these four families.

Methods: Diagnosis was made according to the El Escorial criteria. Genomic DNA was extracted following standard procedures. Mutation analysis of SOD1 gene was carried out by single strand conformational polymorphism (SSCP) analysis followed by direct sequence of samples with abnormal migration patterns. A haplotype study was performed using eight polymorphic microsatellite markers flanking the SOD1 locus. Microsatellites were amplified by fluorescent polymerase chain reaction (PCR) primers and analysed with an automated sequencer.

Results: Clinical phenotype was characterized by early upper motor neuron (UMN) and lower motor neuron (LMN) involvement. This occurred in lower limbs in five out of six patients and in upper limb in one out of six patients, with rapid spread to other limbs, appearance of bulbar signs within 1 year and death a few months later. In two of six patients, atypical signs (sexual, behavioural and urinary disturbances) were also found. Mean age at onset was 47.3 years and mean duration of disease, for four deceased patients, was 12 months. The G41S mutation has been previously reported in four patients from one US family presenting with a similar rapidly progressive course, but not further clinically described. All the six FALS patients showed a common haplotype covering 1.05 Mb genomic region surrounding the SOD1 gene.

Discussion and conclusions: The clinical phenotype in all patients was quite uniform and characterized by spinal onset and a very short survival. All the six FALS patients shared the same haplotype, therefore suggesting a founder effect origin for the G41S mutation. In view of this result, we propose that all the patients derive from a common ancestor.

P25 AGE OF SOD1 A4V MUTATION CAUSING ALS AND FOUNDER EFFECT

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Background: The dominant form of amyotrophic lateral sclerosis (ALS) with exclusively lower motor neuron disease was first described by Sir William Osler in 1880 in the Farr family in Vermont. In the descendents of the Farr family an alanine to valine (A4V) mutation at codon 4 in exon 1 of the SOD1 gene has been described and shown to be responsible for 50% of SOD1 mutations associated with ALS in North America. This mutation is rare in Europe. The explanation for such patterns lies in the demographic histories of populations including effects of genetic drift, migration and natural selection.

Objectives: We aimed to estimate the age of the A4V mutation and to search for a founder effect.

Methods: Ninety-six patients with confirmed A4V mutation and 96 healthy control subjects were genotyped for 14 SNPs across a 21cM region (12cM centromeric to SOD1 and 9cM telomeric). High-throughput SNP genotyping

was performed using *Taq*man assay in 384-well format on the ABI prism 7900HT sequence detection system (Applied Biosystem). Haplotype frequencies and association statistics for the polymorphisms were estimated using Haploview version 3.2; *p*-values less than 0.05 were considered statistically significant. We used a Bayesian method (using Markov chain Monte Carlo method) for multipoint linkage disequilibrium mapping incorporated in the program DMLE+ version 2.2 to estimate the age of A4V.

Results: Five SNPs located between 1cM centromeric and 2cM telomeric to SOD1 were associated with A4V ALS. A single haplotype consisting of three SNPs associated with A4V (p = 4.87e-10). Using haplotype frequency data for these three SNPs, the estimated age for A4V is 63.5 generations (~1270 years). Haplotypes for all five SNPs estimated the age to be 76.8 generations (~1536 years).

Conclusions: SOD1 A4V descended from a single founder 1200 to 1600 years ago.

P26 A NOVEL ASN86LYS MUTATION IN SOD1 CAUSES RAPIDLY PROGRESSIVE AUTOSOMAL DOMINANT AMYOTROPHIC LATERAL SCLEROSIS WITH EARLY RESPIRATORY FAILURE

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Background: Autosomal dominant familial amyotrophic lateral sclerosis (FALS) is most frequently associated with point mutations in the Cu/Zn-superoxide dismutase (SOD1) gene on chromosome 21. The corresponding changes in the structure of the SOD1 protein leads to increased oxidative stress by lipid and protein peroxidation, to increased frequency of protein aggregates and to alterations in sensitivity for proapoptotic stimuli such as increased Ca^{2+}, Fas and/or TNF sensitivity which results in degeneration of cortical and spinal motor neurons.

Objectives: A 63-year-old male presented a rapid course of a clinically definite ALS with predominantly lower motor neuron signs, severe respiratory distress but no signs of bulbar involvement. Family history revealed unclear early deaths accompanied by severe respiratory distress in the maternal line and a further case of rapid progressive respiratory failure with one sister of the index patient.

Methods: Disease progression was monitored at 3-month intervals by detailed clinical examination including FVC; functional deficits were estimated by the ALSFRS-R. The individual results were compared with pooled data from a local database. Genomic DNA was obtained from whole blood samples. The coding region and the flanking intron sequence of all five exons of the SOD1 gene were amplified by polymerase chain reaction (PCR). Single strand conformation polymorphism (SSCP) analysis was used as a screening technique and the PCR product of the conspicuous exon 4 was sequenced.

Results: Clinical follow-up showed a much more rapid disease progress in our index patient compared to the control ALS population. Genetic analysis revealed a heterozygous T to A exchange at nucleotide position 1067 in the coding region of exon 4 of the SOD1 gene resulting in an amino acid substitution of lysine for asparagine at codon position 86 (Asn86Lys).

Conclusion: A novel Asn86Lys base exchange appears to be associated with a rapid disease course and early respiratory involvement. These results expand the spectrum of known ALS associated mutations in the SOD1 gene.

P27 THE RARE EXON 4 MUTATION G93D IN THE SOD1 GENE IS ASSOCIATED WITH A SLOWLY PROGRESSIVE AND LOW PENETRANCE FORM OF ALS

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Background: The finding of rare, unknown DNA mutations in SOD1 linked to FALS is not infrequent, and a common problem for genetic counselling is poor understanding of the genotype-phenotype correlation. During the study of 12 FALS patients, we characterized a G93D (GGT > GAT) mutation in exon 4 of the SOD1 gene. This mutation has only been described in two patients, one of them of Italian origin.

Objective: To obtain a better knowledge of clinical features associated with G93D, including age at the onset of symptoms, penetrance, variation of expression and progression.

Family description: The proband is a 45-year-old female, who developed a slowly progressing muscular atrophy in lower limbs, with widespread fasciculations but without pyramidal signs. An EMG confirmed a diffuse neurogenic pattern in all limbs. Since the patient referred that her grandmother was affected by similar disturbances, an accurate family history was collected. The proband's paternal grandmother died at the age of 75 years after three years of progressive motor impairment, starting in lower limbs, without pyramidal signs. A proband's second cousin (the daughter of her grandmother's sister), born in 1938, had a diagnosis of

ALS in 1984 and is still alive. Both the proband's father and her cousin's mother, who were obligate carriers of the gene, did not develop ALS. During pre-test genetic counselling, the proband was informed about the meaning and the limits of the molecular tests and an informed consent was obtained.

Methods: Blood samples were collected and DNA purified using Perfect gDNA blood mini kit. Prior to sequencing the five coding regions of the SOD1 gene, at least 100 nt of flanking introns were analysed with DHPLC. To screen for homozygous changes, an equal quantity of normal amplicon of the five exons of the SOD1 gene was added to the PCR of the patient before heteroduplex formation. Direct sequencing of the DNA fragments showing an abnormal chromatographic profile was performed using dideoxynucleotides method with the Big Dye kit (Applied Biosystems) on an ABI Prism 3100 automated sequencer and analysed with the software 'Factura' and 'Sequence Navigator'.

Results: Sequence analysis revealed a G > A point mutation at nucleotide position 1087 in the heterozygous state in the proband, which is the same mutation found in her cousin. The mutation was absent in 121 SALS and 11 FALS cases, and in 130 healthy controls.

Discussion: The triplette GGT coding for glycine at codon 93 is a hot spot site for mutation, since it has all possible mutations in any position, giving rise to six different amino acid substitutions. For the majority of them the SOD1 activity seems quite normal and the resulting phenotype is very mild, usually slowly progressing and with an incomplete penetrance.

P28 DIFFERENT CLINICAL PHENOTYPES IN MOTOR NEURON DISEASE CAUSED BY DYNACTIN 1 (DCTN1) GENE MUTATIONS

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Different motor neuron diseases (MND) show selective vulnerability of motor neurons. A common pathomechanism is not yet known. Axonal transport seems to play an important role in the underlying mechanism. Genetically proven changes in proteins involved in axonal transport may be a basis for defined MND, e.g. amyotrophic lateral sclerosis (ALS). Based on perceptions from axonal transport in mouse model studies, it is generally accepted that mutations in the multiprotein complex dynactin are possible susceptibility factors or candidates for developing motor system degeneration. Furthermore, single MND patients have been reported with mutations in the p150 subunit of the dynactin-1-gene (DCTN1) as the underlying reason for their disease. We investigated the p150 subunit of the DCTN1 gene in 552 consecutive adult MND patients. We found different missense mutations in exons 7, 13, 15, 20, 27 and 31 and mutations in intron 2 and 28 as possible disease causing factors. None of the mutations were found in healthy controls (n = 160). In addition, we detected one polymorphism in exon 13. Analysis of phenotypes in MND patients with DCTN1 gene mutations showed a broad spectrum. We observed classical ALS patients as well as atypical MND patients with exclusive lower motor neuron affliction or uncommon clinical signs and additional abnormal brain MRI findings. In nearly all the patients, the disorders started in the upper limbs. The age at onset, individual disease progress and main clinical characteristics showed no consistent pattern in our patients.

We show that mutations in the DCTN1 gene can be associated with motor neuron degeneration, but with respect to a variable clinical phenotype, additional possible underlying pathophysiological mechanisms or other genetic or external factors have to be discussed.

P29 TRINUCLEOTIDE REPEAT EXPANSION DETECTION AT SCA2 LOCUS IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Spinal cerebellar ataxia 2 (SCA2) is an autosomal dominant disorder caused by CAG repeat expansions in the *SCS2* gene. The clinical phenotypes of SCA2 patients are heterogeneous. Most patients show clinical symptoms of cerebellar ataxia; other features sometimes include manifestations of Parkinsonism and motor neuron degeneration. Typically, individuals with SCA2 possess one normal SCA2 allele (14–31 CAG repeats) and one unstable expansion of the CAG repeat (>31 repeats: usual range 35–77 repeats). CAG repeat expansion at the SCA2 locus has been recognized recently as an uncommon cause of Parkinsonism both in familial and sporadic Parkinson's disease (PD). The repeat lengths in those symptomatic PD cases ranged from 33 to 43. To date, SCA2 repeat lengths have not been well characterized in ALS patients. Because there is a suggestion of motor neuron compromise with SCA2 expansions, we have investigated SCA2 repeat lengths in ALS cases.

Objective: To investigate the trinucleotide repeat range of the *SCA2* gene in familial and sporadic ALS cases.

Patients and methods: A total of 534 ALS cases (381 sporadic, 153 familial) and 418 controls were

studied using polymerase chain reaction (PCR) analysis. The amplified products of all samples were analyzed on both 3% agarose gels and 6% denaturing polyacrylamide gels.

Results: The trinucleotide repeats length of the SCA2 CAG domains in these 534 ALS patients range from 15 to 34 (predominantly 22 and 23 repeats, allele frequencies 91% and 5%, respectively). The SCA2 repeat length in 418 controls ranged from 17 to 32 (again predominantly 22 and 23 repeats, allele frequencies 93% and 4%, respectively). In 534 ALS patients, repeat lengths > 26 occurred in 29 chromosomes, including five cases showing borderline expansions (32, 32, 33, 34, 34 repeats respectively). In 418 controls, repeat lengths > 26 occurred in 5 chromosomes, with only one case showing a borderline expansion (32 repeats).

Discussion: We conclude that there is no significant difference between ALS and control DNAs with respect to the most common SCA2 repeat length. However, the numbers of repeat lengths > 26 and borderline expansions are increased nearly five-fold in ALS cases. These data suggest that SCA2 expansions warrant further investigation as factors that may confer increased susceptibility to ALS.

P30 MUTATIONAL RECOVERY OF VIRAL OPEN READING FRAMES IN SELECTED HERV-K-RELATED ENDOGENOUS RETROVIRUSES IN ALS PATIENTS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease (MND) characterized by motor neuron degeneration in motor cortex, brainstem and spinal cord. The molecular pathogenesis and etiology for sporadic ALS remain unknown. Previously our group demonstrated elevated antibody reactivity to an endogenous retrovirus (HML-2/Herv-K) in patients with sporadic ALS. Other groups have found elevated reverse transcriptase activity in ALS patient plasma. The particular human endogenous retroviruses (HERVs) that are transcribed in ALS patients are unknown. It is also unclear how HERV proteins could be produced given the many inactivating mutations that are present in almost all endogenous retroviruses in the human genome.

Objective: To determine the type and quantity of endogenous retroviruses that are transcribed in the peripheral blood cells of ALS patients and controls.

Method: Whole blood was obtained from 17 ALS patients and 9 healthy controls and processed immediately into total RNA. Reverse transcriptase PCR (RT-PCR) with degenerate primers was employed to amplify the polymerase region of all HERV-K related RNAs. Subsequent amplifications used primers specific for the gag and protease region of a particular subset of Herv-K related viruses. RT-PCR products were cloned and sequenced. Sequencing data were analyzed using Vector NTI software.

Results: Greater than 95% of the RNA produced by HERVs in ALS patient or control blood samples was derived from five integrated proviruses. Sporadic ALS patients had a significantly increased nucleotide mutation rate in HERV RNAs homologous to one of these five proviruses (located in human chromosome 8p) relative to healthy controls. Further analysis of RNAs derived from the 8p and related loci found that 24% of the 17 sporadic ALS patients had mutations at up to four different nucleotides in the protease gene that corrected a premature termination of the full protease open reading frame (ORF). In contrast, similar mutations were not detected in any of the nine healthy controls.

Conclusions: Individuals with sporadic ALS exhibit an elevated rate of mutation in RNAs homologous to a particular integrated HERV in chromosome 8p. In 24% of the patients, these mutations would allow for the translation of full length viral gene products that could not be transcribed from the 8p HERV sequence integrated in the human genome. These results help explain appearance of an immune response to HERV proteins in ALS patients. The elevated mutation rate of HERV 8p related RNAs is consistent with reverse-transcriptase mediated replication of this HERV in some sporadic ALS patients.

P31 PESTICIDES, PARAOXONASE AND SPORADIC ALS

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Background: Sporadic ALS (SALS) appears to have an increased incidence in Gulf War veterans (1), in people exposed to agricultural chemicals or living in rural areas, and in airline pilots (2). All of these are exposed to organophosphates. Organophosphates are hydrolyzed by the enzyme paraoxonase 1 (PON1). Certain polymorphisms in the promoter and exons of the PON1 gene can increase or decrease the activity and expression of PON1 (3). Any impairment of PON1 could allow organophosphates to attack motor neurons.

Objectives: To investigate the interactions between PON1 polymorphic genotypes and pesticide exposure in SALS.

Methods: PON1 promoter SNPs (−909c > g, −832g > a, −162g > a and −108c > t) and coding SNPs (L55M and Q192R) were studied in 143 SALS cases and 143 controls matched for age, sex and ethnicity. Alleles, genotypes and haplotypes were compared between

SALS cases and controls. Logistic regression was used to investigate gene-environment interactions.

Results: The frequency distribution of the −108c > t allele was different in cases and controls ($p = 0.03$). All four promoter genotypes were associated with SALS. Of interest, the high-expression variants −108c > t CC (OR = 0.12, CI 0.03−0.46, $p = 0.01$) and −162g > a AA (OR = 0.04, CI 0.01−0.29, $p = 0.01$) were negatively correlated with SALS. The low-expression haplotype at these SNPs was weakly associated with SALS. The coding L55M and Q192R genotypes and haplotypes were not associated with SALS. An analysis of gene-environment interaction showed that people with PON1 susceptibility alleles at each of the six SNPs, who were also exposed to pesticides, were even more likely to have SALS than if they had the susceptibility alleles alone (ORs between 1.81 and 2.39).

Discussion and conclusion: Promoter genotypes that control the expression of the PON1 enzyme were associated with SALS. In particular, the −108c > t polymorphism, which has a large effect on PON1 expression, may be important. The interaction of the PON1 alleles and pesticide exposure was only slightly increased compared to allele susceptibilities alone, implying that genetic susceptibility is the major component underlying this association.

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P32 GENOTYPE AND EXTREME EXERCISE – A LETHAL COMBINATION IN ALS?

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Background: Athleticism has been identified as a possible risk factor in ALS. Large scale case controlled epidemiological studies have failed to confirm this observation. Confounders in such studies have included overmatching of controls, and failure to stratify ALS patients by gender, age of onset, and site of onset. Hypoxia-inducible factors including vascular endothelial growth factor (VEGF) and angiogenin have been implicated in the pathogenesis of ALS. 'At-risk' alleles have been identified in VEGF in some populations.

Objective: To conduct a detailed case controlled study of lifetime metabolic activity in ALS patients and controls, and to genotype the ALS population with respect to 'at-risk' alleles in VEGF and angiogenin.

Methods: Over 100 ALS and matched controls completed a detailed questionnaire about their lifetime history of exercise and metabolic expenditure. The disease and control populations were divided into cohorts comprising those with low, medium, high (>1 standard deviation from the mean) and extreme (>2 standard deviations from the mean) histories of lifetime metabolic expenditure. Disease and control populations were genotyped for 'at-risk' angiogenin and VEGF haplotypes.

Results: There was a disproportionately high number of males with spinal onset ALS who had a history of high or extreme metabolic expenditure. The −2578C homozygous VEGF genotype was identified in 70% of individuals with high or extreme energy expenditure (EEE), compared to 29% of ALS patients with medium energy expenditure, and 22.6% of controls. This genotype has been previously reported to be associated with increased risk of early age of onset. The −2578C, −1154G and −634C haplotype was identified in 70% of patients with a history of EEE, compared to 22% of patients with medium expenditure, and 18% of controls. Sixty percent of patients who were −2578C homozygous also carried the angiogenin 'at risk' G allele of rs11701 SNP.

Conclusions: Exercise may combine as an environmental susceptibility in ALS patients with VEGF and angiogenin 'at risk' haplotypes.

P33 ANALYSIS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) HAPLOTYPES AND RISK FOR ALS IN NORTH AMERICAN, IRISH AND SCOTTISH POPULATIONS

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Background: VEGF has been implicated in the pathogenesis of ALS. In a meta-analysis of over 900 ALS patients and more than 1000 controls from northern Europe, individuals homozygous for the VEGF haplotypes (−2578A/−1154A/−634G/ or −2578A/−1154G/−634G/) had a 1.8 times greater risk for the development of ALS. These at-risk haplotypes were associated with reduced VEGF expression and lowered serum VEGF levels.

Objectives: We sought to examine these at-risk haplotypes in the North American, Irish and Scottish populations.

Methods: We examined the VEGF haplotypes in a total of 466 sporadic ALS (SALS), 77 familial ALS (FALS) (no SOD1 mutations) and 408 matched control samples.

Results: Analysis of the North American population identified an increase in frequency of the −2578C, −1154G and −634C alleles in SALS and FALS compared to controls ($p > 0.024$). However, there was no significant difference in the distribution of the previously identified at-risk VEGF haplotypes in SALS/FALS compared to controls ($p > 0.05$). Analysis of the VEGF haplotype in the Irish population showed no significant difference between SALS individuals and controls ($p > 0.36$). Further analysis of the Irish population demonstrates a trend towards increased frequency of the −2578C, −1154G and −634C alleles in specific subgroups. However, combined analysis of the North American, Irish and Scottish populations showed no significant difference in the at-risk VEGF haplotype when SALS were compared to controls ($p > 0.3$).

Conclusions: There is increasing evidence strongly suggesting a biological role for VEGF as a modifier of motor neuron degeneration. However, this study does not replicate the haplotype association identified in a large cohort of Northern Europeans. Other haplotypes may be important in conferring risk in subpopulations of ALS.

P34 ASSOCIATION BETWEEN HFE MUTATIONS AND ALS

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Background: Oxidative stress is considered to play a key role in the process leading to motor neuron degeneration. Iron is a very potent pro-oxidant and its metabolism can be disrupted due to mutations in the *HFE* gene. Recent studies suggested HFE mutations to be a risk factor for various other diseases, including Alzheimer's disease. This prompted investigators to study the relationship between HFE mutations and the development of ALS. One study showed that an H63D mutation was associated with an increased risk of developing ALS.

Objectives: To investigate the possible association between the presence of a C282Y or an H63D HFE mutation and the development of ALS. Furthermore, to determine a possible effect of HFE mutations on disease characteristics.

Methods: Two hundred and eighty-nine ALS patients were randomly selected. Genotyping was performed for both the C282Y and the H63D mutations. Controls were taken from two population based studies carried out among individuals living in the same region. A random sample of 5886 individuals were genotyped for both HFE mutations. Logistic regression analysis adjusted for known risk factors was performed.

Results: We found that homozygous mutations at H63D were independently associated with an increased risk of developing ALS (OR = 2.16, $p = 0.02$). The remainder of genotypes were not associated with ALS. The presence of the C282Y or H63D mutations did not significantly affect site of first weakness or survival. However, carrying an H63D mutation was associated with a higher age at onset (OR = 0.73, $p = 0.033$).

Discussion: One study reported a significant association between HFE mutations and the development of ALS. However, this was based primarily on H63D heterozygotes. In our study of larger sample size, only H63D homozygosity was significantly associated with an increased risk of ALS. The difference in genetic background of the population and the fact that we used a large cohort of population-based controls could account for these differences. These findings suggest a role of HFE mutations in development of ALS, although the underlying mechanism is still uncertain. A possible association with iron overload is the subject of a future study.

P35 SOD1 DOWN-REGULATION IN ALS PATIENTS CARRYING AN HFE MUTATION

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Background: *Hfe* mutations are associated with an increased risk of iron loading in the body, which in turn is associated with an increased risk of oxidative stress and altered inflammatory reactions. We have reported that 31% of sporadic ALS patients carry an *Hfe* mutation compared to only 14% of non-ALS patients with some type of neuromuscular complaint ($p<0.005$). The worldwide prevalence of *Hfe* mutations in the general population is reportedly 10–12% which is similar to that reported for the non-ALS group. The prevalence of this mutation is the second highest genetic mutation ever identified within the ALS population. Of the two most common types of *Hfe* mutation, the H63D mutation is the specific *Hfe* mutation associated with ALS.

Objectives: Our hypothesis is that *Hfe* mutations establish a permissive cellular environment that will enable genetic factors that promote the induction of ALS.

Methods: To test this hypothesis, we developed two stable cell lines from a human neuronal cell line (SH-SY5Y) that carry either the H63D mutation or C282Y mutation of the *Hfe* gene. We determined SOD1 expression by Western blotting and mitochondria membrane potential (MMP) using JC-1 staining. We also examined SOD1 expression in human muscle biopsy samples obtained during standard diagnostic procedures for ALS. Only those individuals with the H63D mutation have been examined because this was the most prevalent mutation in the ALS samples.

Results: The *Hfe* mutant cell lines had a decrease in SOD1 expression and in particular the level of SOD1 in the H63D cell line is lower than the cell line carrying the C282Y mutation or the wild-type control cell line. In addition, the greatest decrease in MMP occurred in the H63D mutant cell line. Based on the cellular analysis, we examined SOD1 expression in the muscle tissue of the ALS patients and found those individuals with ALS that carry the H63D mutation ($n = 14$) have one-third less SOD1 expression ($p < 0.05$) in muscle tissue compared to ALS patients with wild type *Hfe* ($n = 18$). The SOD1 expression in the muscle biopsies was nearly identical to the results for the *Hfe* transfected cells.

Conclusions: These data suggest that those individuals with ALS who carry an *Hfe* mutation have an increase in oxidative stress compared to individuals with ALS who do not have this mutation. The data also suggest the cell lines provide a novel and reliable system for identifying the mechanism by which the H63D mutation affects ALS. Studies are ongoing to identify additional biomarkers that distinguish between ALS patients with and without the *Hfe* mutation. This information is important for understanding how *Hfe* mutations are a risk factor for ALS and for developing intervention strategies.

Acknowledgement: This research was supported by the Muscular Dystrophy Association.

P36 DETOXIFICATION GENE POLYMORPHISMS AND SUSCEPTIBILITY TO SPORADIC MND IN A RUSSIAN POPULATION

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Background: A significant pathogenic factor of motor neuron disease (MND) may be oxygen free radicals, and detoxification is an important metabolic process in which free radicals might be formed and subsequently transformed into non-toxic products.

Objectives: We studied polymorphisms in the genes of detoxification systems in ALS patients and controls.

Materials and methods: We studied a 96bp insertion polymorphism of the cytochrome *2E1* gene (CYP2E1*1D), CYP2D6*4 polymorphism of the cytochrome *2D6* gene, deletion polymorphism of glutathione-S-transferases T1, M1 and P (GSTM1, GSTT1 and GSTP), and slow-rapid acetylation polymorphism of N-acetyltransferase type 2 gene (*NAT2*) in 75 patients with sporadic MND within the age range 28–72 years and 105 randomly sampled controls from Moscow. Analysis was performed using PCR and autoradiography of PCR products, with lengths corresponding to allelic variants of the named genes. The diagnosis of MND was made according to revised El Escorial criteria (1998); rapid (>10 degrees per six months), moderate (5–10 per six months) and slow (<5 per six months) progression rates were designated according to loss of Norris ALS Score degrees.

Results: A considerably increased frequency of CYP2E1*1D was observed in MND patients (14% versus 2.5% in controls; $p < 0.001$). Analysis of genotype distributions did not identify the CYP2E1*1D/CYP2E1*1D genotype in the control group. Comparative analysis of genotype distributions showed statistically significant differences between the patients and controls in our population ($p = 0.0018$). The GSTT1(0/0)/GSTM1(0/0) and GSTT1(+)/GSTM1(0/0) genotype combinations prevailed among the controls ($p = 0.033$) and GSTT1(0/0)/GSTM1(+) and GSTT1(+)/GSTM1(+) combinations prevailed among MND patients. In contrast, the analysis for CYP2D6, GSTT1, GSTP1 and NAT2 gene polymorphisms has revealed no differences in distribution of different genotypes and alleles between patients and controls. There were no associations found between polymorphic variants and clinical features of MND (ALS/PBP, progression rates).

Conclusion: These data suggest that the CYP2E1*1D allele is associated with sporadic MND and is involved in the pathogenesis of sporadic MND in Russian patients, being possibly associated with production of higher levels of toxic metabolites of xenobiotics of Phase I of detoxification.

P37 GENETIC RISK FACTORS FOR SPORADIC AMYOTROPHIC LATERAL SCLEROSIS (SALS)

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Background: ALS is the neurodegenerative disorder of still unknown aetiology. The mutations of SOD1 have been shown as a risk factor of familial ALS (FALS). Polymorphisms of different genes involved in the processes associated with possible mechanisms of motor neuron degeneration due to ALS, may play a role as risk factors of ALS.

Objective: To study the polymorphisms of the genes that are potentially involved in the pathogenesis of SALS, i.e. inflammation (interleukin-1 −511 C/T, interleukin-6 −174 G/C polymorphisms, SERPINA3 A/T polymorphism); oxidative stress (paraoxonases (PON) Gln192Arg PON1 Leu55Met and PON2 Cys311Ser and metalloproteinase-9 C/T polymorphisms).

Material and methods: We included 167 patients with definite or probable diagnosis of SALS and 452 healthy controls matched for age and sex. The diagnosis of ALS was established according to El Escorial criteria (1994). The FALS cases were excluded on the basis of the positive

family history of ALS. The polymorphisms were studied using PCR technique and restricted enzyme digestion.

Results: The study showed that among all studied polymorphisms the distribution of the following polymorphisms differs between ALS cases and controls: PON1 Gln192Arg polymorphism [cases (n = 166): Gln/Gln – 72, 47.6%; Gln/Arg – 67, 40.4%; Arg/Arg – 20, 12.0% vs. controls (n = 440): Gln/Gln – 242, 55%; Gln/Arg – 167, 38%; Arg/Arg – 31, 7%, p = 0.032] , PON2 Cys311Ser polymorphism [cases (n = 166): Cys/Cys – 17, 10.2%; Cys/Ser – 71, 42.8%, Ser/Ser – 78, 47%, controls (n = 397): Cys/Cys – 28, 7.1%, Cys/Ser – 133, 33.5%, Ser/Ser –236, 59.4%, p = 0.006], and SERPINA3 A/T polymorphism only in bulbar onset SALS [cases: (n = 43): CC –7, 16.3%, CT – 19, 44.2%, TT – 17, 39.5%, genotype distribution in controls (n = 404): CC – 106, 26.2%, CT – 193, 47.8%, TT – 105, 26.0%, p = 0.05].

Conclusion: Our findings suggest that among studied polymorphisms only those related to oxidative stress may be involved in the risk of SALS.

P38 METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISMS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: The N5, N10-methylenetetrahydrofolate reductase catalyses the remethylation of homocysteine to methionine and the biosynthesis of nucleotides. Two polymorphisms, C677T and A1298C of the *MTHFR* gene have been identified, and associated with cardiovascular diseases, neurovascular, neurodegenerative diseases, psychiatric disorders and cancer. Amyotrophic lateral sclerosis (ALS) is a chronic progressive devastating disease of the central nervous system, characterized by the death of upper and lower motor neurons.

Objectives: The aim of the study was to determine whether the MTHFR C677T and A1298C polymorphisms were genetic risk factors for ALS in Turkey.

Results: A total of 52 ALS patients and 278 controls were genotyped for the MTHFR C677T and A1298C polymorphisms. The 677T allele frequency of the *MTHFR* gene was 30.77% in the ALS patients and 35.97 in the healthy controls. The 1298C allele frequency of the *MTHFR* gene was 37.5% in the ALS cases and 35.43% in the controls. The MTHFR C677T genotype showed a 1.9-fold increase risk for ALS (OR = 1.916; 95% CI 1.033–3.554) χ^2 = 4.351; df = 1; p = 0.037). Likewise the C677T/A1298C compound genotype showed a 2.4-fold increase risk for ALS (OR = 2.434; 95%CI 0.996−5.952; χ^2 = 4.013; df = 1; p = 0.045).

Discussion: To our knowledge, this is the first evidence showing an association between the MTHFR polymorphisms and ALS. Although we only analysed 52 sporadic ALS patients and 278 healthy controls, the association is only significant in the genotypes C677T and C677T/A1298C. However, there was no allele association. To clarify the data, more ALS patients should be studied in the future.

P39 MUTATION SCREENING OF THE *VAPB* GENE IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a heterogeneous, progressive, degenerative disease characterized by loss of motor neurons in the spinal cord, brain stem and motor cortex. To date, five genes have been found to be associated with familial ALS (FALS). A mutation in exon 2 (166, C→T) of vesicle-trafficking protein *VAPB* gene has been found in seven families with diagnoses ranging from typical ALS to mild spinal muscular atrophy (SMA) (1). Their common clinical presentation is an autosomal dominant slowly progressive disorder characterized by fasciculations, cramps and postural tremors. Historical data indicate that the seven families have a common Portuguese ancestor and probably belong to a single large family.

Objective: To screen the *VAPB* gene for mutations in sporadic ALS (SALS).

Methods: DNA from 90 sporadic cases and one familial case was screened for mutations in *VAPB* gene. Primers were designed by primer 3 software to amplify all six exons plus the 5' and 3' untranslated regions. The products were analyzed by dideoxy termination sequencing on a Beckman coulter 8000. The sequences were aligned by Sequencher V4.2 and analyzed by eye. Each variant identified was also analyzed among 100 control subjects.

Results: We detected the known mutation, C to T at nucleotide 166 (Pro56Ser) in the familial ALS case which is from the same geographical region as the reported families. The same mutation was not found in SALS cases.

Discussion: Mutations in the *VAPB* gene are not a common cause of sporadic ALS.

Reference

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P40 POLIO VIRUS RECEPTOR-RELATED TYPE 2 (*PVRL2*) GENE IS ASSOCIATED WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, age-dependent, neurodegenerative disorder of motor neurons with both sporadic and familial forms. The cause of most types of ALS is unknown and the disease is untreatable. We have recently identified chromosome 19q13 as a candidate susceptibility locus in sporadic ALS (SALS).

Objective: To identify susceptibility polymorphisms in candidate genes on chromosome 19q13 for sporadic ALS.

Methods: This association study of a North American Caucasian population included 14 polymorphisms spanning PVRL2 and TOMM40 in 128 parent-child triads (trios) and 192 SALS patients, age and gender matched with 192 control subjects. PVRL2 functions as a herpes virus receptor and TOMM40 is an outer membrane protein of the mitochondria that helps import nuclear coded proteins into the mitochondria. High-throughput SNP genotyping was performed using *Taq*man assay in a 384-well format on the ABI prism 7900 sequence detection system (Applied Biosystem). Haplotype frequencies and association statistics for the polymorphisms were estimated using Haploview version 3.2. *P*-values less than 0.05 were considered statistically significant.

Results: The polymorphism rs3745150, located in the intergenic region between PVRL2 and TOMM40, demonstrated an association with SALS ($\chi^2 = 5.172$, 1df, $p = 0.023$) in the case-control model. The association was borderline in the trio sample ($\chi^2 = 3.279$, 1df, $p > 0.05$). However, the polymorphism rs2927466, located in intron 2 of PVRL2, was over-transmitted to affected offspring ($\chi^2 = 4.149$, 1df, $p = 0.0417$). None of the TOMM40 polymorphisms showed an association in either the case-control or the trio model.

Conclusion: PVRL2 polymorphisms associated with SALS in the case-control and trio groups. This study lends support to the hypothesis of neuronal vulnerability associated with polymorphisms in molecules utilized by viruses for their entry.

P41 A PHENOTYPIC-GENETIC STUDY OF NINE POLISH MEN WITH SPINAL BULBAR MUSCULAR ATROPHY (SBMA)

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Background: SBMA (Kennedy's disease) is a rare, adult form of X-linked recessive neurodegenerative disorder caused by the expansion of a polymorphic trinucleotide CAG-repeat sequence in the first exon of the androgen receptor (*AR*) gene. The CAG repeat within the *AR* gene is polymorphic in healthy individuals, ranging in size from 5 to 33 repeats. In SBMA patients, however, the CAG repeat ranges in size from 40 to 62 repeats. Classically, patients are presented with proximal spinal and bulbar weakness and atrophy, generalized fasciculations, sensory involvement and slow progression.

Methods: We examined nine males (from seven families) with clinical phenotype of SBMA and three female carriers from two families. The patients underwent the standard neurological examination and neurophysiological studies. Serum levels of creatine kinase (CK) and hormones (testosterone, LH, FSH, PRL) were measured. DNA was extracted from peripheral blood according to standard procedures for banking. The detection of a pathologically expanded CAG sequence in the *AR* gene was performed by polymerase chain reaction (PCR) techniques.

Results: Male patients (mean age: 45.1 $\pm$ 13.1 years; 20–70 years) presented the history of progressing distal(8/9) or proximal (1/9) limb and facial muscular weakness with orofacial fasciculations (9/9), amyotrophy of different distributions, nasal voice (9/9), dysphagia (9/9), hand tremor (5/9), distal peripheral sensory disturbances (2/9) and gynecomastia (8/9) as well as impotence (8/9). The 'quivering chin' phenomenon occurred in 7/ 9 men. There was no evidence of upper motor neuron involvement. One of the examined female carriers presented with a 30-year history of fasciculations and minimal distal weakness and cramps in the legs, while the remaining two were asymptomatic. DNA analysis revealed expanded size of CAG repeats in Xq11-12 in the *AR* gene in all males (range 26–52 CAG repetitions) and in females (range 46–48 CAG repetitions). Neurophysiological studies showed signs of chronic denervation in all males studied. There was no correlation between CAG repetition size and the age of disease onset and duration. However, we found significant reverse correlation between CAG repetition length and level of testosterone and prolactin.

Conclusions: The correlation between the repeat length and the severity and earlier onset of the disease has been often described in the literature; however, we did not find this correlation in the patients studied. Our patients are presented with less common phenotype of SBMA with distal muscle weakness.

P42 SPG3A MUTATION SCREEN IN A COLLECTION OF NORTH AMERICAN HEREDITARY SPASTIC PARAPLEGIA CASES

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Background: The hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by progressive lower limb spasticity and weakness often associated with bladder disturbance (1). Identification of 11 HSP genes has shown that several pathophysiological pathways are involved in this disease including impairment of axonal transport, a common link with other neurodegenerative diseases (2). The gene mutated at the SPG3A locus encodes atlastin, a protein localized to the golgi apparatus (3). To date over 20 mutations have been identified in individuals with early onset HSP. These include missense changes and a frame shift mutation that leads to a slightly truncated protein.

Objectives: To determine the frequency of SPG3A mutations in a collection of 70 North American HSP cases and to characterize the nature of these mutations.

Methods: Primers were designed to amplify the 14 exons and intronic flanking sequence. The amplicons were analyzed by dHPLC WAVE and the variants were sequenced. The control samples were tested by direct sequencing. Standard Western blot and RT PCR analysis were performed on lymphoblast cell line extracts.

Results: We identified one segregating variant in a French Canadian family that was absent in 80 control samples. This variant leads to an in frame deletion of N436, a predicted glycosylation site (3). Western blot analysis of the patient sample did not show an altered migration pattern of atlastin, but revealed a reduction in protein quantity. RT PCR analysis suggests that this stems from a reduced atlastin mRNA level in lymphoblasts.

Conclusion: Our data suggest that the N436 site does not affect glycosylation and that this mutation causes HSP by haplo-insufficiency. Additional quantitative studies and development of a zebra fish knockdown model are underway to better understand the role of atlastin in maintenance of healthy corticospinal tract neurons.

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P43 ACQUIRED NUCLEIC ACID CHANGES MAY TRIGGER SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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This presentation brings together evidence to support the hypothesis that acquired nucleic acid changes are the proximate causes, 'triggers', or 'initiators' of sporadic amyotrophic lateral sclerosis (ALS). Clinical features that support this hypothesis include: focal onset and spread, and the individualized rate of progression. Clues from the epidemiology of sporadic ALS include the increase in its age-specific incidence with age, suggesting accrual of time-dependent changes, increased frequency of monoclonal gammopathy of uncertain significance in patients with ALS, suggesting shared risk factors, and the emergence of smoking, having a known carcinogen, as its first 'more likely than not' exogenous risk factor. The identification of any exogenous risk factor suggests that a large proportion of sporadic cases have a triggering mechanism susceptible to that factor. Ingestion of the products of *Cycad circinalis* has been hypothesized to be implicated in causing western Pacific ALS. *Cycad* contains both neurotoxic factors and carcinogens. The dissimilarity between western Pacific ALS and neurotoxic diseases suggests greater likelihood that the effects of DNA alkylation are its proximate cause. Evidence in support of a hypothesis does not constitute proof of the hypothesis, but sets the stage for its further consideration (1).

(1) Muscle Nerve 2005 In Press.

P44 INCIDENCE OF ALS IN BELGRADE, 1992–2004 PRELIMINARY DATA

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Background: Most of the epidemiological studies of ALS have reported an increased incidence in the last three decades.

Objectives: To estimate the incidence of ALS in the population of Belgrade during the period of 1992–2004.

Methods: ALS cases were collected by analysing hospital in- and out-patient registers at the Institute

of Neurology, which is the national referral neurological centre, and in departments of neurology in an additional three clinical centres in Belgrade. The El Escorial diagnostic criteria for ALS were applied to all cases enrolled in the register. Each patient was regularly followed up during the disease. The incidence rates were calculated by standard procedures and the calculation of confidence intervals was based on Poisson's frequency distribution for rare events.

Results: In the period l992–2004, 244 (150 male and 94 female) patients with ALS were identified in The District of Belgrade. The mean age of onset was 59.5 $\pm$ 11.2 (range 25–87) years. The overall average annual incidence rate of ALS was 1.2/100,000 (95% CI 0.9–1.4); 1.5/100,000 (95% CI 1.1–2.0) for males, and 0.9/100,000 (95% CI 0.7–1.1) for females. The highest age-specific incidence rate was registered in the age group 60–64 (3.5/100,000). The incidence rate for spinal onset of ALS was 0.85/100,000, and for bulbar onset 0.25/100,000. During the observed period the incidence rate of ALS in Belgrade showed a statistically significant increasing tendency ($y = 0.783 + 0.058x$, $p = 0.047$).

Conclusion: In comparison to our previous data (1) the results suggest a significant increase in the incidence of ALS in Belgrade.

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P45 RISING PREVALENCE IN 2004 IRISH ALS POPULATION

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Background: The Irish ALS Register was established in 1993 and has full case ascertainment from 1995 onwards. During the last decade there have been rapid advances in the treatment of ALS including improved utilization of gastrostomy nutrition, the introduction of non-invasive ventilation and the availability of riluzole.

Objectives:

1) To determine the incidence and prevalence of ALS in Ireland during the period 1995–2004.
2) To examine the temporal trend of ALS in Ireland by comparing the incidence and prevalence in the three-year period 1995–1997 with the three-year period 2002–2004.

Methods: The Irish ALS Register collects information on all patients diagnosed with ALS in Ireland using multiple sources of information.

Results: The average annual incidence for the three-year period 2002–2004 was 2.1 per 100,000 person-years and 2.9 per 100,000 person-years among the population older than 15 years. This is almost identical to the incidence figures reported for 1995 –1997 (2.1 and 2.8 per 100,000 person-years). In contrast, the crude prevalence on 31 December 2004 was 7.6 per 100,000 population and 10.2 per 100,000 population over the age of 15 years. These figures were statistically significantly higher than the prevalence figures on 31 December 1996, namely 4.7 per 100,000 of the total population (z test = -1.93, $p = 0.053$), and 6.2 per 100,000 for the population older than 15 years (z test = -2.22, $p = 0.026$). The median survival of an individual diagnosed with ALS in 2004 is longer than that for a patient diagnosed in 1996.

Discussion: The prevalence rate of ALS in Ireland has risen by 65% between 1996 and 2003, but the incidence rates have remained unchanged over the same time period.

Conclusion: This finding is due to improved prognosis among Irish ALS patients and may reflect the improved availability of multi-disciplinary healthcare for this patient group.

P46 RILUZOLE AND ALS SURVIVAL: A POPULATION-BASED STUDY IN SOUTHERN ITALY

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting motor neurons, for which there is no effective cure. Riluzole is to date the only treatment that prolongs ALS survival, as evidenced by two clinical trials. However, results on the efficacy of riluzole in observational population-based studies with a longer follow-up are conflicting and, therefore, it is still unclear if the effect of the drug is limited to an early stage of the disease and to some specific subgroups of patients, such as subjects with younger age or with bulbar onset.

Objectives:

1) To evaluate the effect of riluzole on ALS survival at two years in a cohort of incident cases.
2) To examine whether bulbar ALS benefits from the medication to a greater extent.
3) To assess the efficacy of the drug in elderly patients.

Methods: Source of the study was a prospective population-based registry of ALS established in Puglia, Southern Italy, in 1997. We examined survival of 126 out

of 130 incident ALS cases that were diagnosed during the period 1998–99.

Results: Seventy-three patients (58%) were prescribed riluzole and the remaining 53 (41%) were not. Riluzole therapy increased survival rates at 12 months by approximately 10% and prolonged survival by six months (18.2 months versus 12.4; peto test; 2.78; $p = 0.09$). This beneficial effect was present among bulbar onset ALS (peto test: 4.11; p, 0.042), but not in subjects with limb onset (peto test: 0.48; $p = 0.4$). In patients aged more than 70 years riluzole treatment was associated with an eight- months longer median survival time (15.4 months versus 7.1; $p = 0.03$) and a reduction in mortality rate at 12 months by 27%, regardless of site of symptom onset. Riluzole use was an independent predictor of survival at 12 months from the diagnosis in this series in multivariate analysis with borderline significance (HR: 0.5; 95% CI 0.2–1.02; $p = 0.06$). Riluzole was effective among cases with bulbar onset ALS (HR: 0.29; 95% CI 0.08 −0.98; $p = 0.05$) and elderly patients (HR: 0.32; 95% CI 0.1−0.99; $p = 0.05$), whereas in subjects with limb onset there was no effect on survival at 12 months (HR 0.72; 95% CI 0.30−1.75). In each model riluzole did not influence survival at 24 months.

Conclusions: In this population-based series, we found that riluzole therapy improves ALS survival. The efficacy of the drug was present among bulbar onset ALS and older patients, but not in subjects with limb onset. The favourable effect of the drug was transient, as it was lost in prolonged follow-up. Our observations support the use of riluzole at an early stage of ALS in bulbar and elderly patients. The appropriate duration of riluzole treatment remains, however, to be established.

SLAP Neurologists: G Belfiore, G Benedetto, N Cacudi, A Cazzato, P Colamartino, P Di Viesti, S Epifani, F Lincesso, B Maggio, V Monitillo, A Moramarco, A Nicolaci, C Nozzoli (Brindisi), Sergio Pasca (Casarano), Rosaria Pulimeno (Gallipoli), Giuseppe Russo, V Santamato, IL Simone, G Strabella, M Terraciano, P Tota, F Valluzzi.

P47 INCREASED RISK FOR DEVELOPING ALS IN THE MILITARY POPULATION: FURTHER EVIDENCE FROM THE FRENCH POPULATION

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Background: Recent works have underlined an apparent increased risk for developing ALS for veterans from the Gulf war and then more globally in the military population. To date, the reasons for such an increased risk remain unclear. It has been supposed that it is due to particular expositions during conflicts. It is also likely that it is simply due to the profession itself and the multiple expositions encountered during the professional course.

Objectives: To determine the percentage of military personnel in an unselected ALS population and analyse the type of activity to try to define some specific exposure.

Methods: Three hundred and eleven consecutive ALS patients followed in our clinic during the last three years were interrogated for their past or present professional activity in the army. When present, the rank (officer or not) and service (air force, marine, ground, gendarme) were also collected.

Results: There were 21 patients belonging to the military population either retired or not, comprising 18 males and 3 females. Characteristics of ALS were not different from classical ALS. The observed frequency was compared to the proportion of military personnel in our country. The relative risk (RR) to develop ALS was 4. There was no influence of the rank of patients. Conversely, patients who worked in the Air Force had a significantly higher risk with a RR of 10.

Discussion: The increased risk for developing ALS in military personnel is also found in the French population. A significant predominance of subjects from the Air Force is noted in our country in contrast to other services. However, this is not a systematic survey of the military population in the whole country or a case control study. On the other hand, we interrogated 311 consecutive patients, without selection. Our region (Languedoc-Roussillon) does not have a significant number of military bases. Thus, this does not seem to represent a possible bias. We are now planning a large national survey to include a systematic questionnaire of ALS patients to identify environmental risk factors. Indeed, military personnel are likely to be exposed to a large variety of potentially neurotoxic factors or suspected risk factors for ALS such as radiation (electromagnetic or ionizing), solvents, heavy metals, vaccinations, infectious diseases, trauma, or physical activity.

Conclusion: An increased risk for developing ALS was found among 311 consecutive ALS patients followed in our MND clinic. This risk is particularly high in those personnel belonging to the Air Force. Work is in progress to confirm these results in the whole country and to identify environmental risk factors in that population.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 91–103

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THEME 3 *IN VIVO* EXPERIMENTAL MODELS

P48 ANALYSIS OF GENE EXPRESSION USING WHOLE GENOME ARRAYS IN NORMAL AND SMA AFFECTED TISSUES

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The loss of motor neurons and muscle atrophy in spinal muscular atrophy is caused by the reduced activity of the survival motor neuron (*SMN*) gene. This gene codes for a protein that has been shown to be required for assembly of large complexes involved in RNA processing, such as the spliceosome and ribonucleo protein (RNP) particles. Such a function for SMN protein does not explain why, when function is reduced, specifically the motor neuron and/or the muscle are affected.

We have created a *Drosophila melanogaster* model for SMA by mutating the endogenous *smn* gene. Reduction of zygotic activity of this gene leads to neuromuscular defects in affected larvae. The characterization of these defects shows that they concentrate at the neuromuscular junction (NMJ) (1). In order to further our understanding of the connection between smn function and the RNA associated processes, we generated RNA samples of smn mutant and normal fly tissues (the genetic background we used as 'normal' is as close to the smn mutant background as possible, except for the offending mutation). Using standard protocols and adhering to strict MIAME guidelines, labelled RNA was produced using these RNAs as templates. Each of these samples was then hybridized to *Affymetrix Drosophila* whole genome arrays (in triplicate or quadruplicate). The resulting hybridization results were normalized employing several different methods. The most reliable of these were used and gene ontology determined for the genes for which expression profiles were changing comparing normal with smn affected. We will discuss the technical background to these experiments as well as the further experimentation to back up the array findings. The most interesting genes that are implicated in this system will be presented.

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P49 ANALYSIS OF TRANSCRIPT TRANSPORT AND TRANSLATION CONTROL BY THE SURVIVAL MOTOR NEURON PROTEIN

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The loss of motor neurons and muscle atrophy in spinal muscular atrophy is caused by the reduced activity of the survival motor neuron (*SMN*) gene. This gene codes for a protein that has been shown to be required for assembly of large complexes involved in RNA processing, such as the spliceosome and ribonucleo protein (RNP) particles. Such a function for SMN protein does not explain why, when function is reduced, specifically the motor neuron and/or the muscle are affected.

We have created a *Drosophila melanogaster* model for SMA by mutating the endogenous *smn* gene. Reduction of zygotic activity of this gene leads to neuromuscular defects in affected larvae. The characterization of these defects shows that they concentrate at the neuromuscular junction (NMJ) (1). In order to further our understanding of the connection between smn function and the RNA associated processes, we generated smn mutant fly ovaries. These tissues have been studied over the last decades specifically because they appear to provide an easy accessible (by genetics) system in which several mRNAs are translocated and translationally controlled in very precise ways. It is also clear that these processes are driven by components of a machinery of a set of highly conserved gene functions.

Smn mutant ovaries develop in ways very similar to ovaries mutant for several other gene functions; these encode for proteins known to associate with either smn function or with RNP function. Several mRNA transcripts that are normally localized to particular positions in the egg chamber are dispersed in smn mutant ovaries. In the normal tissues, the translocation of such transcripts is coupled to their translation inhibition. This system allows the transport of the transcripts to a particular position; translocation is controlled to allow translation to take place only when they have arrived at their appropriate place. This generates not only localized protein function but also controlled high concentrations of proteins in these regions. This coupled translocation and translation control process seems disrupted in smn mutant ovaries. Premature translation leading to mis-localized protein is observed. Our findings will be discussed in the light of a role for smn in transcript control at the NMJ.

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ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045014

P50 THE SLOW WALLERIAN DEGENERATION GENE (*WLD*^{S}) DOES NOT PREVENT APOPTOSIS OF MOTOR NEURON CELL BODIES

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Background: The slow Wallerian degeneration gene (*Wld*^{S}) delays the degeneration of injured axons ten-fold and delays axonal death in some non-injury disorders by several weeks, most notably in the progressive motoneuronopathy mouse (*pmn*). Interestingly, motor neuron cell death was also delayed in *Wld*^{S}/*pmn* mice, suggesting either that these motor neurons die secondarily to axon degeneration or that *Wld*^{S} has a direct neuroprotective effect on motor neuron cell bodies as well as on their axons. The question of whether *Wld*^{S} has a direct protective effect on neuronal cell bodies has previously been addressed only in sympathetic neurons *in vitro*, where *Wld*^{S} did not prevent apoptosis. However, it is not clear whether those data hold for motor neurons *in vivo*.

Objectives: Thus, we tested the hypothesis that *Wld*^{S} can directly protect motor neurons whose axons have been almost completely removed by intravertebral avulsion. We also tested for a delay in apoptotic motor neuron death following neonatal nerve injury in *Wld*^{S} rats.

Methods: Intravertebral avulsion is one of a number of difficult surgical techniques where the effect of *Wld*^{S} can now be studied following our development of a *Wld*^{S} rat model. L4 ventral roots were avulsed in three-month-old rats and motor neuron survival assessed in cresyl violet (Nissl stained) 25 μm cryostat sections 3–4 weeks later. Neonatal nerve injury was made by crushing sciatic nerve in P3 rats and motor neuron survival assessed after 3–14 days.

Results: Eighteen percent and 16% of motor neurons survived in *Wld*^{S} rats three and four weeks, respectively, after intravertebral avulsion. These numbers were similar to wild-type controls. The motor neuron survival rate after neonatal nerve injury was also little altered. Forty percent of motor neurons survived after three days and 30–35% after 7–14 days.

Conclusions: We conclude that *Wld*^{S} has no direct neuroprotective effect on motor neuron cell bodies *in vivo*. Thus, the increase in motor neuron survival in *Wld*^{S}/*pmn* mice most probably shows that motor neuron death in the *pmn* mutant is secondary to axon death. Our data show how *Wld*^{S} can be used as a tool to test whether various ALS models bring about motor neuron death directly or whether this death is secondary to axon degeneration. The data also support the model of compartmentalised cell death programmes in motor neurons and show how the availability of *Wld*^{S} rats has made possible new surgical studies in this field.

P51 A NEW MOUSE MODEL FOR MOTOR NEURON DISEASE: THE DOUBLE HETEROZYGOTE CRA1/SOD^{G93A} MOUSE

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Background: Familial forms of ALS are associated with mutations in the gene for the Cu/Zn superoxide dismutase (SOD1). These mutations gave rise to several animal models, the G93A (Gly ->Ala) mutation being most commonly used. Transgenic mice expressing the mutated human Cu/Zn superoxide dismutase (SOD1^{G93A}) develop motor neuron pathology and clinical symptoms similar to those seen in patients with ALS. Another model for motor neuron disease is the *Cra1*/+ mouse. A missense point mutation in the motor protein dynein, leading to impairment of retrograde axonal transport, results in progressive motor neuron degeneration in *Cra1*/ heterozygous mice, without major reduction in lifespan.

Objectives: The objective of this study was to compare the phenotype of double heterozygote *Cra1*/SOD^{G93A} mice to hemizygote SOD1^{G93A} mice with regard to motor activity, survival time and weight, expecting a more severe phenotype because of the added defect of dynein. Furthermore, we compared the number of motor neurons in the ventral horn of the spinal cord between both groups.

Methods: *Cra1*/+ females (background C3), obtained from Ingenium Pharmaceuticals AG, Martinsried, Germany were crossed with SOD1^{G93A} males (background B6), obtained from The Jackson Laboratory, Maine, USA. F1 offspring (*Cra1*/SOD^{G93A}, SOD1^{G93A}) was used for investigations. *Cra1*/+ and +/+ wild-type mice served as controls. Motor activity was measured by Rotarod. Survival, weight, and Rotarod data were analysed statistically. For histological investigations, mice were perfused with 4% paraformaldehyde. Cervical and lumbar spinal cord were dissected and processed according to standard protocols. Slices (10 μm) were prepared and stained with cresyl violet (Nissl staining).

Results: Surprisingly, the double heterozygote *Cra1*/SOD^{G93A} mice showed a statistically significant extension of lifespan and improved motor activity compared to hemizygote SOD1^{G93A} mice. Moreover, the loss of body weight of *Cra1*/SOD^{G93A} mice was reduced compared to SOD1^{G93A} mice during disease progression.

Conclusions: Our data suggest that an impairment of retrograde fast axonal transport leads to an attenuation of the mice SOD^{G93A} phenotype. Further studies will be necessary to reveal the molecular mechanisms of these findings.

P52 CONTRIBUTION OF BACKGROUND STRAIN TO PHENOTYPE IN G93A MUTANT SOD1 TRANSGENIC MICE

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of the motor system which occurs in sporadic and familial forms. Mutations in Cu/Zn superoxide dismutase (SOD1) were identified in affected individuals from a subset of familial ALS kindreds; this has led to the creation of transgenic mice which overexpress one of several mutant human SOD1 genes. The G93A mSOD transgenic mice exhibit an autosomal dominant, adult-onset, progressive neurodegeneration of the motor system which bears a striking resemblance to ALS, both clinically and pathologically. These mice have been extensively utilized to test hypothetical mechanisms of motor neuron death and putative therapeutic agents.

Objectives: We hypothesize that genetic susceptibility combined with specific lifestyle elements, such as exposure to environmental toxins, or diet, contribute to the development of sporadic ALS. There is considerable evidence for the contribution of modifying genes and genetic background to variation in phenotypes in the mouse. We are constructing and characterizing several different mouse congenic lines carrying the human mG93A SOD1 transgene as the first step in identifying genes which can modify the course of motor neuron degeneration.

Methods: The mSOD1 trait has been bred onto DBA, HeJ, and B10 backgrounds. Transgene copy number is verified in all mice in collaboration with G. Alexander et al. (2004) (1). Beginning at 50 days of age, mice are monitored for body weight and motor skills (grip strength, hind limb splay, righting reflex, Rotarod performance, and gait). To examine the progression of cellular and molecular changes which accompany the development of motor symptoms in the three background strains, cells are isolated from frozen spinal cord sections using laser-capture followed by quantitation of specific gene expression by real-time RT-PCR.

Results: We have observed that genetic background, and in some strains, gender, influences the phenotype of motor neuron degeneration in the G93A mice. Some changes in motor function (grip strength and Rotarod performance) already vary significantly between the three strains at 50 days. However, onset of motor symptoms does not correlate with ultimate survival in each mouse strain. We have found the shortest survival and most rapidly progressive disease course, as well as gender dependence of the phenotype, in the HeJ mice. Among genes whose expression levels are being quantitated, we find that specific to late stage motor neurons in HeJ mice is the significant up- regulation of the Ca^{+2} -permeable GluR3 AMPA receptor subtype, and decreased expression of the GluR2 editing enzyme, ADAR2.

Discussion and conclusions: Our finding of increased susceptibility in the HeJ mice is similar to what has been reported by Heiman-Patterson et al.(2) or the G93A phenotype on the SJL mouse background. The up-regulation of the GluR3 receptor subunit in degenerating motor neurons is consistent with our previous work establishing the role of Ca^{+2}-permeable AMPA receptors in mutant SOD1-mediated motor neuron death. It is also consistent with the findings of Spalloni et al.(3) and Rembach et al. (4) in *in vitro* and in whole spinal cord studies of AMPA receptor subunit expression in G93A transgenic mice.

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P53 MOUSE MOTOR NEURON DISEASE CAUSED BY TRUNCATED CU/ZN SUPEROXIDE DISMUTASE

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Background: Mutation of Cu/Zn superoxide dismutase (SOD1) contributes to a portion of the cases of familial amyotrophic lateral sclerosis (FALS). We previously reported on a FALS family whose members had a mutant form of SOD1 characterized by a 2-base pair (bp) deletion at codon 126 of the SOD1 gene (Leu126delTT). We also reported the possibility that this mutation made the mutant SOD1 protein functionally unstable and, as a result, the amount of mutant protein was considerably low. In order to investigate the cellular consequences of the mutation, we produced a transgenic animal that

expressed the same mutated form of SOD1 as found in this family.

Objectives: We produced transgenic mice that were introduced with normal and mutated copies of human SOD1 gene: wild-type SOD1 (W), wild-type SOD1 with a FLAG epitope at C terminal (WF), mutated SOD1 with the 2-bp deletion (D), and SOD1 with the 2-bp deletion with FLAG (DF). Among these heterozygotes, those that constantly exhibited motor neuron symptoms were inbred to obtain homozygotes. Then heterozygous and homozygous mice were evaluated further.

Methods: Mice were evaluated by clinical assessment (onset of the disease and date of death), biochemical analysis (SOD1 activity, Western analysis and northern analysis) and pathological examination (haematoxylin-eosin and Klüver-Barrera stain as well as immunohistochemistry using anti-GFAP antibody, anti-SOD1 antibody and anti-FLAG antibody).

Results: The mice heterozygotic for the human mutated SOD1 (D and DF) showed distinct ALS-like motor symptoms, whereas the mice heterozygotic for the normal SOD1 (W and WF) mice did not. Homozygotes of D and DF lines showed ALS symptoms at an earlier age and died earlier than the heterozygotes. By northern analysis, the mRNAs for all human SOD1s were confirmed in these lines. All the human SOD1 proteins, except the D mutant, were detectable by immunoblot. The D protein was only confirmed when it was concentrated by immunoprecipitation. Neuropathologically, loss of spinal motor neurons and reactive gliosis were common features in the symptomatic lines. The remaining motor neurons in these mice exhibited Lewy body-like eosinophilic inclusions that were positive for SOD1. In the case of DF lines, inclusion was also positive for FLAG.

Discussion and conclusions: The biochemical and pathological characteristics of these mice were quite similar to those of human FALS patients with the same mutation. It remains to be solved why the mutant SOD1 protein is quite low in quantity, with the existence of hyaline inclusions that are positive for the mutated form of SOD1. This intriguing mouse model will provide an important source of information of the pathogenesis of FALS.

P54 PROTEIN NITRATION IN A TRANSGENIC MOUSE MODEL OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background: Multiple mechanisms have been proposed to contribute to Cu/Zn superoxide dismutase (SOD1)-linked familial amyotrophic lateral sclerosis (ALS) pathogenesis, including oxidative stress. Early evidence came from the identification of markers of oxidative stress in the cortex and spinal cord of patients with sporadic and familial ALS. Among these markers, nitrotyrosine (NT) has attracted attention in view of Beckman's theory, which suggests a greater propensity of SOD1 mutants to use peroxynitrite as an enzyme substrate, leading to tyrosine nitration. However, no comprehensive study on the protein targets of nitration in ALS has been reported.

Objectives: To understand the role of protein nitration in ALS pathogenesis and identify the protein targets, we carried out a proteome-based analysis of spinal cords from transgenic (Tg) SOD1 G93A mice at a presymptomatic stage of the disease.

Methods: Proteins from spinal cord tissue of nine-week-old Tg G93A SOD1 mice and age-matched Tg wild-type SOD1 mice, were separated by two-dimensional (2-D) electrophoresis. For the identification of nitrated proteins, samples were subjected to 2-D Western blot (WB) analysis probing the membrane with anti-NT antibody. Nitrated proteins in 2-D WB were matched to the Coomassie-stained gel and excised from the gel for the identification using MALDI mass spectrometry.

Results: We found an increased level of NT-immunoreactivity in spinal cord protein extracts of Tg G93A SOD1 mice at a presymptomatic stage of the disease compared to age-matched controls. NT-immunoreactivity is increased in the soluble fraction of spinal cord homogenates and is found as a punctuate staining in motor neuron perikarya of presymptomatic ALS mice. Using a proteome-based strategy, we identified the nitrated proteins under physiological or pathological conditions and compared their level of specific nitration. Alpha and gamma enolase, ATP synthase beta chain, heat shock cognate 71 kDa protein and actin were over-nitrated in presymptomatic ALS mice. In addition, we identified by MALDI mass spectrometry 16 sites of nitration in proteins oxidized *in vivo*. Alpha enolase nitration at Tyr43, also a target of phosphorylation, represents an additional indication of the possible interference of nitration with phosphorylation.

Discussion and conclusions: We propose that protein nitration may have a role in ALS pathogenesis, acting

directly by inhibiting the function of specific proteins and indirectly interfering with protein degradation pathways and phosphorylation cascades.

Acknowledgement: This work was supported by Telethon Foundation and Cariplo Foundation.

P55 PROTEOMIC CHARACTERIZATION OF AGGREGATES IN A TRANSGENIC MOUSE MODEL OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Background: Ubiquitinated and Cu/Zn superoxide dismutase (SOD1)-immunopositive protein inclusions in spinal cords are a histological and biochemical hallmark of disease progression in familial ALS (FALS). The aggregates are found in the cytoplasm and cannot be dissociated with strong detergents or reducing agents. Furthermore, in murine models of ALS aggregates can be detected even before the onset of clinical symptoms. It is still controversial if the inclusion bodies may have a cytotoxic activity, are secondary inoffensive products or have a protective role by capturing abnormal proteins.

Objectives: To investigate the role of protein aggregation in FALS pathogenesis, we characterized the proteins that co-aggregate with mutated SOD1 in a transgenic mouse model of FALS, which over-express human SOD1 carrying the G93A mutation (Tg SOD1 G93A).

Methods: Experiments were carried out on Tg SOD1 G93A mice at a late stage of the disease. Age-matched Tg mice overexpressing wild-type human SOD1 and non-Tg littermates were used as controls. Proteins were separated by two-dimensional electrophoresis (2-DE) and the gel maps were compared by computerized image analysis. Two-thirds of each sample was used for Coomassie-stained 2-D gels and one-third for Western blotting. The most abundant proteins present in aggregates of Tg SOD1 G93A mice were identified by MALDI mass spectrometry.

Results: The total amount of proteins was about two times more in sick mice than in healthy mice. Among them, structural proteins, chaperones, and enzymes involved in energy metabolism were identified. In agreement with previous results accumulation of mutant SOD1 occurred selectively in the spinal cord of SOD1 G93A mice with the observation of multimeric forms. The accumulation of ubiquitinated or polyubiquitinated proteins was only detected in mice carrying the mutation and consistent anti-ubiquitin immunoreactivity was evident especially at high molecular weights. Carbonylated proteins were also found only in tissues of Tg SOD1 G93A mice.

Discussion and conclusions: Using a proteomic approach we have characterized the proteins present in FALS aggregates. The proteins identified are involved in axonal transport, folding, energetic metabolism, and are highly ubiquitinated or carbonylated only in SOD1 G93A mice. This confirms a large number of impaired proteins in aggregates and suggests a strong oxidative situation and a decreasing efficiency of the ubiquitin proteasome system.

Acknowledgement: This work was supported by Telethon Foundation and Cariplo Foundation in Italy.

P56 DETERGENT INSOLUBLE FORMS OF SOD1 ARE DISTRIBUTED TO BOTH OUTER AND INNER MEMBRANES OF MITOCHONDRIA AND ARE ASSOCIATED WITH DEVELOPMENT OF ALS IN TRANSGENIC MOUSE MODELS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive paralytic disorder caused by degeneration of the upper motor neurons of the motor cortex in the brain, and lower motor neurons in the brainstem and spinal cord, resulting in progressive wasting and paralysis of voluntary muscles. Mutations in SOD1 are found in 20% of familial ALS. Transgenic and knockout mouse models demonstrated that mutant SOD1 exhibits a toxic property that is associated with development of ALS. How mutant SOD1 exhibits neuronal toxicity is not known.

Methods: Multiple lines of transgenic mice that over-express different SOD1 mutations at different levels were developed. These mice were characterized by using clinical, genetic, biochemical and morphological studies including immuno-electron microscopy. The fractions from outer membrane (OM), inner membrane (IM), intermembrane space (IMS), and matrix were analyzed with F-SOD1 and different mitochondrial markers. The fractions from OM and IM were washed three times with 0.1% NP40 solution. The soluble SOD1 (mouse and human) in the cytosol was also analyzed as a positive control (S3). Fractions from IMS and matrix were not washed with detergent because such washing would remove both signals of the mitochondrial markers and SOD1; suggesting SOD1 in these fractions is soluble.

Results: Some of the mice showed ALS-like phenotype and pathology, and others did not when the expression of mutant SOD1 was low. Taking advantage of the single and double transgenic mice that showed different expression level, different phenotypes and pathology, we found that both mutant and wild-type SOD1 had a similar distribution pattern in the affected and unaffected tissues, and in the different subcellular organelles. However, only the

detergent insoluble form of SOD1 ($SOD1^{DIS}$) is associated with development of ALS. $SOD1^{DIS}$ was distributed to both the outer and inner membrane of mitochondria, leading to mitochondrial damage.

Discussion: Using transgenic mouse models expressing different forms of SOD1 and a crossbreeding strategy, we observed multiple lines of evidence showing that ALS associated toxicity of SOD1 is derived from a detergent-insoluble form, rather than soluble form of SOD1. ALS-associated mutant SOD1 is able to convert wild type SOD1 from soluble form to insoluble form, which in turn exacerbates the disease, a property similar to that found in prion disease: $SOD1^{DIS}$ is distributed to both inner and outer membranes of mitochondria. Both mutant and wild-type SOD1 confer ALS associated toxicity as long as they form $SOD1^{DIS}$.

P57 MITOCHONDRIAL ALTERATIONS IN ASYMPTOMATIC G93A-SOD1 TRANSGENIC MICE (SOD1-TG)

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Background: Mitochondria abnormalities are consistently found in human ALS and in SOD1-TG mice. Mutant SOD1 was found in the mitochondrial matrix of SOD1-TG mice (1). SOD1 also binds and docks to BCL2 and forms aggregates that preferentially interact with spinal cord mitochondria (2). However, the nature of mitochondrial damage and the selective vulnerability of motor neurons in ALS are still matter of debate. Moreover, mitochondrial alterations are seen also in wobbler motor neuron disease mice (3) at early stages of the pathology and in human sporadic ALS.

Objectives: Evaluation of abnormalities in energy metabolism, calcium buffering ability, modulation of mPTP in spinal cord (and brain) mitochondria from asymptomatic SOD1-TG mice (5–10 weeks of age).

Methods: Mitochondria isolated on Percoll gradients were challenged with activators or specific inhibitors of mPTP opening (4,5). The ability to accumulate and release calcium was measured indirectly with CG5N and also mitochondrial activities were assayed (3).

Results: Spinal cord mitochondria from 10-week-old SOD1-TG mice showed lower activities of complex IV ($p<0.0001$) and I ($p<0.03$) and exhibited a lower threshold for Ca^{2+}-induced swelling than control ones. Both in 5- and 10-week-old SOD1-TG mice, mitochondria had significantly higher capacity to accumulate calcium (in 30 s) and reduced calcium release upon collapsing $\Delta\Psi_{\square}$ with an uncoupler of oxidative phosphorylation. Thapsigargin (5 μM) greatly increased the proportion of mitochondria undergoing mPTP opening ($p<0.003$) and the efflux of calcium ($p<0.002$) but it was less effective in SOD1-TG mitochondria.

Discussion and conclusions: Alteration of mitochondrial calcium handling is present in the spinal cord but also in the brain of asymptomatic SOD1-TG mice even at five weeks of age. The selective vulnerability of motor neurons in the pathology may depend on their peculiar micro-environment and on intrinsic properties that may confer to mitochondria a crucial role in the modulation of calcium signalling in these cells.

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P58 THE SELECTIVE CYTOCHROME C OXIDASE DEFICIENCY IN CNS OF G93A-SOD1 MICE IS NOT RELATED TO CYTOCHROME C

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Rodents transgenic for mutant human Cu/Zn SOD (SOD1) develop progressive skeletal muscle atrophy, paralysis and death similar to human cases. The early onset of mitochondrial degeneration suggests that mutant SOD1 causes dysfunction and structural changes in mitochondria, but a detailed mechanism has not yet been established. In the current work we determined the putative involvement of the respiratory chain in brain and muscle mitochondria of day 40 and day 90 G93A-SOD1 mice. We found in brain mitochondria of day 40 SOD1 mice a selective decrease in complex IV enzyme activity, long before any symptoms arose. In line with this finding brain mitochondrial cytochrome spectra indicated a decrease in cytochromes aa_3 but not in cytochromes b and $c+c_1$. Furthermore, day 90 SOD1 (G93A) mice brain and muscle mitochondria showed additionally decreased complex I and aconitase enzyme activities. Interestingly, in comparison to non-transgenic mice mitochondria-associated SOD1 activity was significantly increased in day 40 and day 90 SOD1 (G93A) mice but also in age matched transgenic wild-type SOD1 with intact mitochondrial function. The effect was CNS specific, because it was not observed in muscle mitochondria. Our results suggest

an early direct impairment of cytochrome c oxidase (complex IV) in brain mitochondria which seems not to be linked to the increase in SOD1 activity, or to a recently proposed alteration of cytochrome c association with the inner mitochondrial membrane. We propose that brain mitochondria with mutant SOD1 have a defect in complex IV, generating elevated levels of reactive oxygen species leading at later stages of the disease to an oxygen-radical mediated decline of complex I and aconitase activities. The observed respiratory chain defect in muscle mitochondria of day 90 SOD1 mice reflects most probably an unspecific phenomenon related to muscle denervation, prior to the decline of motor performance.

P59 BIOLOGICAL SIGNIFICANCE OF P38 MITOGEN ACTIVATED PROTEIN KINASE ACTIVATION IN MUTANT SOD1^{G93A} INDUCED MOTOR NEURON DEATH

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the selective loss of motor neurons. In 20% of these familial cases, mutations in the superoxide dismutase 1 gene (SOD1) have been reported. To date, no effective treatment is available and the pathogenic mechanism remains enigmatic. We have previously reported minocycline to significantly attenuate mutant SOD1-induced motor neuron degeneration and to expand the life span of mutant SOD1 overexpressing mice, a mouse model for human ALS. It is thought that the effect of minocycline is exerted through an inhibition of the activation of the p38 mitogen activated protein kinase (MAPK) pathway, which is known to occur in microglia and motor neurons of mutant SOD1 mice.

Objectives: In the present study, we aimed to characterize the biological relevance of up-regulation of p38 MAPK for motor neuron degeneration in the mutant SOD1 mouse.

Methods and sesults: We found the p38 MAPK to be abnormally activated in the ventral part of the spinal cord of the mutant SOD1^{G93A} mice, where significantly increased levels of phosphorylated p38 MAPK were found. The therapeutic effect of minocycline was accompanied by a significant attenuation of this activation. Activated phospho-p38 MAPK was exclusively present in microglial cells and motor neurons. *In vitro* studies showed minocycline to inhibit microglial activation by lipopolysaccharide. In addition, both minocycline and SB203580, a specific p38 MAPK inhibitor, inhibited mutant SOD1-induced apoptosis in cultured motor neurons. We therefore tested whether semapimod, a specific p38 MAPK inhibitor that readily crosses the blood-brain barrier, affected mutant SOD1-induced motor neuron death. Mutant SOD1^{G93A} mice were treated with semapimod or placebo from 70 days of age. Treatment with semapimod effectively inhibited p38 MAPK activity, as phosphorylation of *tau* at residue Thr231 was significantly reduced. It significantly enhanced survival of mutant SOD1 ^{G93A} mice and attenuated motor neuron death in these animals. However, its effect was clearly smaller than that of minocycline.

Conclusion: Our data suggest the p38 MAPK pathway plays a significant, albeit limited, role in mutant SOD1 induced motor neuron degeneration. As the effect of treatment of mutant SOD1 mice with semapimod is smaller than that observed with minocycline, the effect of the latter drug is likely to be mediated by other effects than inhibition of the p38 MAPK pathway alone.

P60 NEUROPATHOLOGICAL CHANGES AND P38 MAPK EXPRESSION IN MOTOR-RELATED AREAS OF THE CENTRAL NERVOUS SYSTEM OF TGSOD1G93A MICE

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Background and objectives: Amyotrophic lateral sclerosis (ALS) is characterized by the selective loss of upper and lower motor neurons; however, many patients with the familial form of ALS (FALS), including those carrying the superoxide dismutase (SOD1) mutations, present only mild or absent upper motor neuron involvement at both clinical examination and autopsy. Transgenic mice carrying the mutant SOD1 recapitulate many of the features of human disease and are therefore extensively studied to investigate the pathogenesis of ALS and to test novel potential pharmacological treatments. In these mice the disease signs have been associated with the degeneration of spinal motor neurons, whereas there is limited knowledge about the involvement of the other upper motor-related areas of the CNS, including red nucleus and reticular formation, both part of the oligosynaptic corticospinal pathway. We have recently shown that both the accumulation of phosphorylated neurofilaments (pNF) and activated p38 mitogen activated protein kinase (p-p38^{MAPK}) are early signs of the degeneration process in the spinal motor neurons of transgenic mice expressing SOD1 with G93A mutation (tgSOD1G93A) and this effect persisted during the progression of the disease. P-p38^{MAPK} was also increased in spinal motor neurons of human sporadic ALS. Thus, we decided to analyse the extent and distribution pattern of these neuropathological markers in the spinal cord, brainstem and sensorimotor cortex of tgSOD1G93A mice during the progression of the disease.

Methods: Immunohistochemical analysis followed by light or confocal microscopy was performed in frozen sections of tgSOD1G93A mice at different stages of the disease and age matched non-transgenic mice, using different antibodies directed to astrocytes, microglia, pNF and activated p38 ^{MAPK}.

Results: We found extensive alterations characterized by motor neuron death, pNF accumulation, reactive gliosis and $p38^{MAPK}$ activation in the cervical spinal cord of this mouse model during the progression of the disease that closely parallels the degenerative changes found in the lumbar segment. In the other motor-related areas, such as red nucleus and sensorimotor cortex, no degenerative changes, reactive gliosis or $p38^{MAPK}$ activation could be observed during the course of the disease.

Conclusion: Our findings show that in this mouse model, similarly to human FALS, the primary disease is driven by the loss of lower motor neurons. Moreover, $p38^{MAPK}$ activation is specifically and selectively expressed in degenerating areas only, confirming its relevant role in motor neuron death. Even with the important species differences in the organization of the corticospinal transmission to motor neurons between humans and rodents, these data further confirm that tgSOD1G93A mice represent a useful model highly necessary to understand FALS and to investigate many of the fundamental mechanisms of lower motor neuron degeneration.

Acknowledgement: This work was supported by Telethon, Italian Ministry of Health, MIUR.

P61 NOGOA UP-REGULATION IN ALS MOTOR NEURONS: INSIGHTS INTO A POTENTIAL FUNCTION IN MOTOR NEURON SURVIVAL

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Background: Amyotrophic lateral sclerosis (ALS) is a motor neuron disease of unknown origin. About 2% of ALS cases are linked to mutations in the gene encoding copper/zinc superoxide dismutase (SOD1). Expression of mutant forms of SOD1 (SOD1m) in transgenic mice leads to motor neuron death and an ALS-like phenotype. Using an unbiased subtractive suppressive hybridization screen, we have identified a clone encoding the neurite outgrowth inhibitor Nogo that is specifically up-regulated in the lumbar spinal cord of asymptomatic G86R transgenic mice. In spinal cord of 90-days-old G86R mice, NogoA mRNA levels are significantly increased compared to the wild-type mice.

Objectives and methods: In order to identify the cell type(s) overexpressing NogoA in the spinal cord of G86R mice and ALS patients, we have performed immunohistochemistry using specific NogoA antibody. In addition, by using a double labelling protocol we have studied the localization of NogoA and its receptor (NgR).

Results: In G86R transgenic mice, in addition to a basal immunoreactivity in glial cells also found in wild-type mice, NogoA is strongly induced in a subset of motor neurons. This result has been confirmed in spinal cord of ALS patients. Furthermore in NogoA positive motor neurons, NgR immunoreactivity was decreased whereas in motor neurons immunoreactive to NgR, NogoA staining was faint or absent.

Conclusion: These results suggest a relationship between NogoA overexpression and NgR down-regulation. The possible implication of this cross-talk in motor neurons death is currently under investigation in *in vitro* models

P62 ROLE OF THE NEURITE OUTGROWTH INHIBITOR NOGO-A IN SKELETAL MUSCLE: IMPLICATIONS FOR ALS

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Background: The pathogenesis of ALS still remains unclear. Growing evidence suggests that initial alterations in skeletal muscle, preceding the onset of disease symptoms, are related to a loss of neuromuscular junction integrity, axonal degeneration and muscle denervation, rather than motor neuron death. Our previous studies showed a characteristic expression pattern of the three major isoforms of the neurite outgrowth inhibitor Nogo (including Nogo-A, -B and -C) in skeletal muscles of ALS patients and SOD1(G86R) mice. We found that the increased levels of Nogo-A and Nogo-B, which had been barely detectable in muscles of control subjects, correlate significantly with the severity of motor impairment of ALS patients, as determined by the clinically validated ALS functional rating scale.

Objectives: We wished to determine the impact of knocking down Nogo-A on the progression of ALS pathology in SOD1(G86R) mice and gain insight into the role of Nogo up-regulation in skeletal muscle.

Methods: We crossbred mice knockout for Nogo-A with mice overexpressing the ALS-related mutation G86R, and followed the survival time. We also performed *in vivo* skeletal muscle transfection of a vector expressing Nogo-A in Thy-1/YFP mice, and looked at the morphology of the neuromuscular junction (NMJ).

Results: Double-transgenic G86R/Nogo-A(−/−) mice survived longer than G86R/Nogo-A(+/+) mice. Transient expression of Nogo-A in skeletal muscle fibres was

sufficient to injure the NMJ by inducing dismantlement of the post-synaptic structures and loss of pre-synaptic terminals.

Conclusions: The ectopic expression of Nogo-A in skeletal muscle initiates a cascade of events leading to loss of NMJs and denervation. This deleterious effect may be relevant to ALS since mice suffering from an ALS-like pathology and lacking Nogo-A live longer.

P63 ALTERNATIVE SPLICING OF PRO- AND ANTI-APOPTOTIC GENES IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Alternative splicing is emerging as a major mechanism of functional regulation in the human genome and it has been involved in many different neurological diseases, including ALS and SMA (spinal muscular atrophy). Change in the balance of pro- and anti-apoptotic Bcl2 family molecules has been described in the motor neurons of human ALS patients as well as in mice expressing mutant SOD1 G93A, the most widely ALS mouse model used (1). Moreover, different members of the Bcl2 family undergo alternative splicing and different isoforms of the same gene could act respectively as pro- or anti-apoptotic.

Objectives: In the present study our aim was to assess if during the progression of ALS there was any change in the splicing isoforms of apoptotic genes that could explain the selective vulnerability of motor neurons.

Methods: *RNase protection assay*. Total RNA was prepared from spinal cord, brain and muscle, of non-, G93A-SOD1 and WT-SOD1 transgenic mice by the guanidinium-thiocyanate method. Total RNA was hybridized with a molar excess of ^{32}P-labelled mouse multiprobe mAPO (Ambion), BFL1, BclX, MnSOD or β-actin riboprobes.

Immunofluorescence. Anaesthetized mice were perfused with paraformaldehyde 4% in PB and the spinal cord removed. Tissues were sectioned (10 μm) on a cryostat. Immunofluorescence was performed according to Ferrr et al. (2).

Results: RNase protection assays on RNA extracted from spinal cord of ALS mice demonstrate that there was not a detectable accumulation of splicing variants for Bcl2, BclX, BclW, Bad, Bax or Bak. Most interestingly we observe the up-regulation of the anti-apoptotic *Bfl1* gene in G93A-SOD1 mice spinal cord, but not in non-Tg or WT-SOD1-Tg, either in the muscle or brain of any genotype. Bfl1 is an antiapoptotic Bcl2 family member and mouse A1 homologue; its expression has been demonstrated to be induced upon activation of the NFkB pathway. The transcriptional induction we observe is not due to a general NFkB up-regulation since we could not detect any increase in BclX or MnSOD mRNA. By immunofluorescence experiments and confocal analysis we demonstrated that Bfl1 localizes in the motor neurons of G93A-Tg mice. It accumulates both in the nucleus and cytosol, where it can form punctate structures.

Conclusions: In the mouse model for ALS we did not observe alterations in the alternative splicing of the apoptotic genes analysed (BCL-2, BCL-X, BCL-W, BAD, BAX and BAK). On the contrary, we observed a transcriptional induction of the anti-apoptotic *Bfl1* gene, in the spinal cord of G93A-SOD1 transgenic mice.

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P64 CONDITIONING EFFECT OF TARGET TISSUE INJURY ON ADULT MOTOR NEURONAL SURVIVAL

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Background: While the critical role played by target tissue in the survival of developing motor neurons is well known, much less is known about its role in the survival of mature motor neurons. This information is vital for the rational design of potential therapies aimed at promoting motor neuronal survival in the adult.

Objective: To determine the conditioning effects of target tissue injury on the survival of axotomised adult motor neurons.

Methods: Adult (6 m) Sprague Dawley rats (n=6 per group) were given three 20 μl injections into the snout of either 1) physiological saline, 2) 0.5% w/v of the myotoxic anaesthetic bupivicaine in saline, or 3) distilled water. After seven days the right facial nerve was avulsed by sustained traction at the stylomastoid foramen in a procedure that produced axotomy at the nerve-rootlet junction. After a further 28 days, rats were perfuse-fixed with 4% paraformaldehyde in 0.1M phosphate buffer. Total numbers of motor neurons in the left and right facial nuclei were estimated in the confocal microscope using a modified optical dissector method.

Results: Approximately 75% of motor neurons were lost by one month after avulsion alone. This was associated with occasional staining of motor neurons by markers of apoptosis such as cleaved caspase 3 and TUNEL. Motor neuron loss in the saline, bupivicaine and distilled water-injected groups was 55%, 49% and 32%, respectively. Compared to the avulsion only group, the neuroprotection afforded by all three forms of muscle damage was significant ($p=0.05$, Mann Whitney *U*-test). Histological examination of snout muscle after four days in all three injected groups showed a qualitative correlation between the degree of lymphocyte infiltration of the muscle and the degree of motor neuron protection conferred.

Discussion: Our results indicate that mechanical damage of muscle due to the injection procedure alone is sufficient to reduce motor neuron loss from 75% to approximately 50%. Adding myotoxic damage has no significant effect on this neuroprotection, but adding osmotic shock reduces motor neurone loss to 32%. The rapid and non-selective cell death induced in muscle tissue by distilled water is probably responsible for the marked lymphocytic infiltration. This peripheral inflammatory reaction may have modified the subsequent retrograde response of motor neurons to avulsion in a direction that favoured their survival.

P65 VEGF RESTORES DECREASED LEVELS OF AKT IN AN ANIMAL MODEL FOR ALS

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Background: Amyotrophic lateral sclerosis is one of the most devastating neurodegenerative disorders. The pathogenesis of this disease that results in a relentless loss of motor function remains unknown. We recently reported that treatment of mutant $SOD1^{G93A}$ overexpressing rats with VEGF resulted in a significant increase in survival compared to the control group. The precise mechanism of the neuroprotective effect of VEGF in ALS remains to be elucidated, but modulation of the phosphoinositol-3-kinase/Akt cascade is one of the most likely targets.

Objectives: In the present study, we aimed to investigate the pathophysiologic role of Akt in ALS and the influence of VEGF on this important anti-apoptotic protein.

Methods and results: Western blot analysis showed that protein kinase B or Akt is lost very early on in the disease. Immunohistological studies showed that this loss is confined to motor neurons, supporting the pathogenetic relevance of the potent anti-apoptotic kinase Akt. *In vitro* transfection experiments with constitutively active Akt revealed the neuroprotective effect of Akt in mutant dependent cyclosporin A-induced cell death in G37R overexpressing N2A cells. To evaluate the relevance of the Akt pathway in the neuroprotective effect of VEGF, Western blot analysis was performed in brainstem homogenates of VEGF-treated $SOD1^{G93A}$ rats. The results showed that treatment with VEGF resulted in a significant increase in the levels of both total and phosphorylated active Akt both after short treatment of 14 days and after chronic treatment.

Conclusions: Our data support the relevance of Akt to the selective loss of motor neurons in ALS and provide evidence for its role as a target of VEGF. Therefore, further development of therapeutic strategies targeting the phosphoinositol-3-kinase/Akt pathway may be useful.

P66 OVEREXPRESSION OF HEAT SHOCK PROTEIN 27 RESCUES MOTOR NEURONS AND IMPROVES MUSCLE FUNCTION FOLLOWING NERVE INJURY IN NEONATAL MICE

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Background: Heat shock proteins (HSPs) are a family of ubiquitously expressed proteins that are up-regulated in response to a range of stresses and play an important role in cellular defence mechanisms. The 27-kDa heat shock protein HSP27 has been shown to have potent neuroprotective effects against oxidative stress and apoptotic cell death. Our previous work has shown that overexpression of HSP27 in transgenic mice significantly reduced cell death in the CNS, possibly through the attenuation of Caspase-3 induction. In motor neurons, HSP27 is also implicated as a survival promoting factor, since exogenous delivery of HSP27 following neonatal nerve injury, enhances motor neuron survival. However, most importantly, it remains to be established whether HSP27 overexpression *in vivo* is able to restore muscle function following neonatal nerve injury.

Objectives: Using transgenic mice that overexpress the human form of HSP27 (hHSP27), we examined whether hHSP27 expression can protect developing motor neurons from injury-induced cell death and improve long term muscle function.

Methods: At birth, the sciatic nerve in one hind limb was crushed in both hHSP27 transgenic mice and wild-type mice. Twenty to 25 weeks later, *in vivo* isometric tension recordings of the EDL muscle were carried out to assess muscle function and motor unit number. The spinal cords and EDL muscles were subsequently removed and processed for sciatic motor neuron counts and morphometric analysis, respectively.

Results: The expression of hHSP27 significantly improved the long-term survival of sciatic motor neurons following neonatal nerve injury. In the wild-type group only 29.8%±5.9% (mean±SEM; $n = 6$) of injured motor neurons survived, compared to 56.4%±2.3% (mean±SEM; $n=6$) in hHSP27 transgenic mice. Importantly, this improvement in survival was reflected by a substantial improvement in muscle function. For example, in the reinnervated EDL muscle of wild-type mice the number of motor units was only 29.1%±1.0% (mean±SEM; $n=6$) compared to 55.1%±5.6% (mean±SEM; $n=6$) in transgenic animals. Furthermore, this increase in motor unit number was associated with improved muscle weight, muscle force, contractile speeds and histochemical markers of EDL muscle activity. Morphometric analysis also revealed an almost normal distribution of muscle fibre sizes in the operated EDL muscle of transgenic mice, whereas in wild-type mice there was a marked increase in the proportion of small atrophic muscle fibres.

Discussion and conclusions: These results show that inducing neonatal motor neurons to express HSP27 protects them against nerve injury-induced cell death. Moreover, HSP27 expression also supported the ability of injured neonatal motor neurons to re-establish functional neuromuscular contacts. Overall, these findings provide further evidence for the therapeutic potential of HSP27 in the treatment of motor neuron diseases.

P67 RILUZOLE/NIMODIPINE/ MINOCYCLINE TRITHERAPY ON RAT ALS MODEL: CONFLICTING RESULTS

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Background: Tritherapy, using a cocktail of three drugs (riluzole, nimodipine and minocycline), has been suggested to have beneficial effects on a mice model for ALS (1). In this work we have tested on the rat ALS model (transgenic rat overexpressing human mutated SOD1 gene) the effect of such a tritherapy on survival time and on clinical scores.

Methods: Rats were divided in five groups (seven rats per group); group I did not receive any drugs, groups II, III and IV received nimodipine, riluzole and minocycline, respectively, and group V a cocktail of the three drugs. Rats were weighed every day. Treatment started at 60 days of age until death. A clinical score using four items (tail test, mobility in the cage, mobility on the table and amyotrophy) with a maximum of 7 points was measured three times/week. EMGs were performed on day 60 for 13 rats, on days 80, 100 and 120 for all animals. Motor neurons from the frontal cortex were counted on paraffin sections after cresyl violet coloration. Sciatic nerve sections (2 micrometres thick) were used for axonal area and axon density measurements. Statistics were made using the Sigma Stat program.

Results: The quantity of drug ingested was measured: rats from group II ingested 25–35 mg/kg/day of nimodipine, group III 18–25 mg/kg/day of riluzole, group IV 64–80 mg/kg/day of minocycline and group V 20–28 mg/kg/day nimodipine, 20–28 mg/kg/day riluzole and 54–68 mg/kg/day minocycline. The analysis of the survival curve indicated that there is no difference between males and females, and no increase in survival rate after any of the treatments. On the contrary, tritherapy and riluzole decreased it. Analysis of the mortality distribution confirmed this result. For male but not female rats, a decrease in weight appeared on day 60 for the rats treated either with riluzole alone or with tritherapy. The axons numbers were significantly diminished after tritherapy. Axon areas were also significantly diminished after tritherapy, riluzole and minocycline treatment. In contrast, nimodipine treatment increased axon areas. Motor neuron counts did not show any significant variation, neither did the electromyography results. Only riluzole improved the clinical scores.

Conclusions: Our results indicated that although riluzole could improve the clinical score and therefore the quality of life, it had adverse effects on the survival rate. This result is important and we believe allows revisiting the beneficial effects of riluzole in human ALS.

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P68 AM1241 SLOWS DISEASE PROGRESSION IN A MOUSE MODEL OF ALS

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Objectives: Effective treatment for amyotrophic lateral sclerosis (ALS) remains elusive. Two of the primary hypotheses underlying motor neuron vulnerability are susceptibility to excitotoxicity and oxidative damage, including inflammatory damage due to microglial activation. CB2 receptor activation blocks β-amyloid induced microglial activation (1). We have previously shown that the cannabinoid Δ^9-THC (which acts on CB1 and CB2 receptors) inhibits both excitotoxic and oxidative damage in spinal cord cultures and that Δ^9-THC slows progression and improves survival in the ALS mouse model ($hSOD^{G93A}$ transgenic mice) even when administered after the onset of disease signs (2). AM1241 is a CB2 selective agonist that has been shown to be effective in models of inflammation and hyperalgesia (3). Thus, we evaluated the efficacy of AM1241 in the ALS mouse model.

Methods: $hSOD1^{G93A}$ mice (B6SJL-TgN [SOD1-G93A] 1Gur) were treated daily beginning on day 75 when

tremors were first observed with 1 mg/kg AM1241 intraperitoneally or vehicle. Motor function was evaluated using a rotarod. The survival endpoint used was the loss of righting reflex within 3 s. The investigator was blinded to the treatment protocol.

Results: Three conditions of ALS, the loss of motor function, paralysis scoring and weight loss, were analyzed using a mathematical model. Loss of motor function (as assessed by performance on a rotarod) was delayed by 12.5 days in male mice by AM1241. In female mice, AM1241 extended performance by three days. Paralysis was scored on a scale from 5 to 0; 5 was healthy, 1 was paralysis. AM1241 extended by five days the time to reach a score of 2.5. AM1241 did not affect weight loss or survival (129.8 ± 1.7 days, vehicle; 129.1 ± 7.0 days, AM1241, $n=16$).

Conclusions: The data presented here indicate that AM1241, a CB2 selective agonist, slowed disease progression in ALS mice. As AM1241 was well tolerated by the animals, CB2-selective compounds may be the basis for developing new drugs for the treatment of ALS.

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P69 DISULFIRAM AS AN INHIBITOR OF SOD1 AND CASPASE-3 IN G86R TRANSGENIC ALS MICE: CLINICAL AND IMMUNOHISTOCHEMICAL EFFECTS

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the selective and progressive degeneration of upper and lower motor neurons in the spinal cord, brainstem, and cerebral cortex. About 10% of ALS cases are familial (FALS). Among the familial cases, 20% are caused by mutations of the Cu/Zn superoxide dismutase (SOD1). The relationship between motor neuron degeneration and the SOD1 mutation is uncertain, but apoptosis is believed to be the mechanism of cell death. The Caspase-3/caspase system is the final common pathway in apoptosis. It is hypothesized that inhibiting the activation of the pathway or the pathway itself would slow cell death and any diseases caused by programmed cell death. Antabuse (Disulfiram) is a potent inhibitor of SOD1 and Caspase-3 as well as other stimulators of the cell death pathway such as TNF-α.

Objective:

1. To determine if disulfiram treatment prolonged life in G86R transgenic ALS mice.
2. To determine if disulfiram influenced the appearance of SOD1 and Caspase-3 in G86R/SOD transgenic ALS mice.

Methods: Four groups of ten G86R mice were fed disulfiram-containing pellets beginning at 50 days after birth. Four different doses (investigator blinded) were administered equivalent to the human dose of 500 mg; 250 mg, 125 mg, and placebo. Two transgenic mice treated with 250 mg and two transgenic untreated mice were sacrificed at 95 days, just prior to the usual time that weakness begins. The spinal cords were removed and tissue sections from one treated and one untreated mouse were selected for single-label immunohistochemistry with antibody to active and proCaspase-3, SOD1. The tissue was washed, mounted on slides and imaged using the Zeiss LSM laser scanning confocal microscope.

Results: All animals were asymptomatic at day 50 when treatment began. Death was the measured endpoint. The mean duration of life for each group was 118 days (placebo), 121 days (125 mg), 144 days (250 mg) and 113 (500 mg). Statistical significance was not achieved but the 250 mg dose showed a trend for increased longevity. Animals receiving 250 mg sacrificed at day 95 showed no expression of Caspase-3, yet it was expressed in the untreated mice. Inactive caspase (activated Caspase-3 precursor) was present in both groups. We observed a difference in cell morphology between the untreated and treated groups when stained with SOD1 but we were unable to draw conclusions.

Conclusion: As Caspase-3 was present in the untreated pre-symptomatic mice but not in the treated asymptomatic mice, our data support the hypothesis that Caspase-3 activation may be inhibited by disulfiram. Further studies are needed to determine how Caspase-3 inactivation, neuronal loss, and clinical weakness are related.

P70 THE EFFECT OF L-CARNOSINE AND ZINC IONS ON SURVIVAL OF G93A/SOD1 MICE

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Background: Mutations of Cu/Zn superoxide dismutase-1 (SOD1) are found in patients with a familial form of amyotrophic lateral sclerosis (ALS). Transgenic mice overexpressing human mutant SOD1 genes, such as G93A, exhibit an ALS-like disease of motor neurons. SOD1 contains sites that bind both copper and zinc, and several studies have reported that administration of excess zinc accelerates death in G93A/SOD1 animals, while more moderate doses may delay death. The dipeptide L-carnosine has zinc-binding as well as anti-oxidant properties. This suggested to us that L-carnosine, given alone or together with zinc, might delay death in G93A animals.

Objectives: We tested the effect of administering L-carnosine, with or without zinc ions, on survival of G93A/SOD1 mice. Our rationale was that the presence of L-carnosine may regulate zinc levels *in vivo*, preventing them from becoming either too high or too low.

Methods: Beginning at age 6–9 weeks, G93A/SOD1 mice were given both L-carnosine and zinc chloride in their drinking water at a daily dose calibrated to correspond to an intake of 3000 mg/kg and 32 mg/kg, respectively, body weight. Motor function was assessed by grading the animals twice weekly on the following scale: 5, healthy; 4, weak hind legs; 3, limping; 2, hind leg paralysis; and 1, death. At the onset of paralysis or when the mice exhibited 10% loss of total body weight the drug was administered daily by i.p. injection, and concentrations of L-carnosine and zinc adjusted according to the total body weight. The animals remained on this regimen until death. The survival endpoint used was the loss of righting reflex within 10 s.

Results: L-carnosine (3000 mg/kg/day) in the presence of zinc (32 mg/kg/day) had a small but insignificant effect on the age at which animals entered stage 2 and on the age at which they died. The sources of water (tap or distilled) had no effect on any of the results. An analysis of the tap water used as vehicle indicated that it had undetectable (less than 0.05 mg/l) levels of zinc.

Conclusions: This is a work in progress. Our studies suggest that L-carnosine in the presence of zinc may have a slight beneficial effect on motor neuron disease in G93A/SOD1 mice. However, further studies are necessary to establish whether it could be effective in prolonging survival of these animals.

Acknowledgements: This study was supported in part by the Forbes Norris ALS Research Foundation.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 104–114

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THEME 4 *IN VITRO* EXPERIMENTAL MODELS

P71 EXPRESSION OF THE CYSTINE/ GLUTAMATE EXCHANGER IN THE RAT BRAIN

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Background: The cystine/glutamate exchanger (antiporter x_c^-) has been suggested to be involved in slow oxidative excitotoxity and in the effects of beta-N-oxalylamino-L-alanine, the molecule responsible for neurolathyrism, a neurotoxic upper motor neuron disease. The x_c^- antiporter is involved in the intracellular transport of cystine, the rate-limiting amino acid in the synthesis of gluthatione, an important anti-oxidant molecule. The mouse cystine/glutamate exchanger has been cloned and shown to be composed of two distinct proteins. One of the two subunits is a novel protein, named xCT, of 502 amino acids with 12 putative trans-membrane domains.

Objective: To study the expression of the xCT protein in the rat brain and spinal cord.

Methods: We have generated polyclonal antibodies to the xCT subunit of the mouse cystine/glutamate exchanger. The antibody was purified on an affinity column. Dot blot and Western blot analyses were performed on protein extracts from rat brain and other tissues using standard techniques. Expression of xCT was studied in several rat brain areas and spinal cord, and in different cultured cells (astrocytes, fibroblasts and neurons) using Western blot and immunocytochemical techniques. Expression of xCT was also studied in several rat tissues (lung, heart, muscle, liver, brain, spinal cord) at different developmental stages (E16, P0, P15, one month, adult).

Results: Peptide pre-adsorption and dot-blot experiments allowed verification of the antibody specificity. Western blot analysis of protein extracts showed that the xCT antibody stained two strong bands of about 38 and 50 kDa in all tissues and cells examined. The two isoforms are expressed in embryonic tissues and they appear to increase during development. Subcellular fractionation indicated that xCT isoforms segregate mainly in the microsomal-mitochondrial fraction. Immunocytochemical analysis showed also a strong staining in neurons and in other cells.

Discussion: We have demonstrated that the xCT protein is expressed in the rat brain and spinal cord as two major isoforms of 38 kDa and 50 kDa, and that its expression is developmentally regulated in all tissues examined. The protein is membrane-bound and also shows a significant expression in embryonic cultured neurons and astrocytes. This represents direct evidence that the x_c^- antiporter might represent an important regulator of the redox processes involving gluthatione in these cells.

The characterization of the biochemical properties and function of the xCT protein in the central nervous system may help to understand the contribution of the cystine/ glutamate antiporter exchanger in the pathogenesis of certain motor neuron diseases.

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045023

P72 DANTROLENE PROTECTS MOTOR NEURONS IN CULTURE AGAINST AMPA RECEPTOR-MEDIATED EXCITOTOXICITY

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Background: Chronic excitotoxicity induced by over-stimulation of calcium-permeable AMPA receptors (AMPAR) was shown to be a key factor in the pathogenesis of amyotrophic lateral sclerosis (ALS). The presence of AMPAR, however, is also necessary for neuronal development. AMPAR may exert such bimodal influence on neuronal survival by altering intracellular calcium homeostasis through control of calcium-induced calcium release from the endoplasmic reticulum (ER).

Objective: The objective of this investigation was to determine a protective effect of the selective ryanodine receptor blocker dantrolene against AMPA/kainate-induced excitotoxicity in cultured motor neurons.

Methods: Ventral horn (VH) neurons and dorsal horn (DH) neurons harvested from E14 rat lumbar spinal cord were cultured for 13 days and then incubated for 24 h with either kainate (30 μM), dantrolene (30 μM) or a combination of both (each 30 μM). SMI32-antibodies against the non-phosphorylated neurofilament-H/M, and tubulin-βIII- antibodies were used to estimate the number of motor neurons and total neuron counts (mean $\pm$ S.E.M.).

Results: Total neuron counts remained equal in each treatment condition (240 $\pm$ 17 VH, $n = 36$; 438 $\pm$ 35.2 DH, $n = 36$). In VH control condition, the percentage of motor neurons in relation to total neuron count was 27% $\pm$ 3% ($n = 9$). Incubation with kainate significantly reduced the percentage of motor neurons to 14% $\pm$ 2% ($n = 8$), $p < 0.01$. Simultaneous application of kainate and dantrolene significantly recovered the percentage of motor neurons to 27% $\pm$ 3% ($n = 9$, $p < 0.01$). Dantrolene alone increased the percentage of motor

neurons present to 40% ± 7% ($n = 7$, $p < 0.05$). This represents a significant increase compared to the kainate condition ($p < 0.01$) and a trend compared to the control condition ($p = 0.07$). The DH preparations contained a low percentage of motor neurons (5.9% ± 2.1%, $n = 12$) where no significant changes due to KA and dantrolene treatment were observed.

Conclusion: Calcium-permeable AMPAR activate ryanodine receptors of the endoplasmic reticulum which causes a spread of the calcium signal along the surface of the ER. The resulting calcium-induced calcium release (CICR) leads to a decrease of ER calcium concentration and to calcium uptake into mitochondria, thereby controlling protein folding and energy metabolism. In our preparation, dantrolene exerted a significant neuroprotective effect against kainite-induced excitotoxicity and in kainate-free conditions where physiological AMPAR activation can contribute to motor neuron death. Dantrolene may positively influence excitotoxic motor neuron degeneration by lowering energy metabolism and protein folding rates, which seem to be pathologically increased in ALS.

P73 NETWORKED SYNAPTIC AMPAR ACTIVATION AND CALCIUM HOMEOSTASIS IN CULTURED MOTOR NEURONS

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Background: AMPA type glutamate receptors (AMPAR) are involved in neuronal development and provide fast excitatory synaptic transmission, but they also mediate cytotoxic insults. Motor neurons are selectively vulnerable towards AMPAR-mediated excitotoxicity, which may play a role in the pathogenesis of amyotrophic lateral sclerosis (ALS). Over-stimulation of AMPAR by non-desensitizing agonists leads to an alteration of intracellular calcium homeostasis in cell culture.

Objective: The objective of this study was to investigate calcium homeostasis in motor neurons under physiological stimulation of AMPAR. Motor neuron-specific disturbances of calcium signals due to physiological AMPAR activation were compared with those of other types of neurons using calcium imaging techniques and patch-clamp recordings.

Methods: Motor neurons (ventral horn) and dorsal horn neurons were harvested from E 14 rat spinal cord and differentiated in a co-culture system with neonatal Schwann cells. Simultaneous patch-clamp recordings at −80 mV membrane potential and high spatiotemporal resolution FURA-2 calcium imaging was used to monitor membrane currents and cytosolic calcium transients in individual neurons.

Results: In cultured ventral horn and dorsal horn neurons, spontaneous inward currents and simultaneous whole cell cytosolic fast calcium transients were observed from DIV 10 onwards, indicating a functional synaptic neuronal network. The calcium signal activity of groups of neurons was tightly synchronized. Inward currents triggered cytoplasmic calcium transients when they exceeded a threshold of ~0.5 nA. These spontaneous inward currents and the corresponding (within 50 ms) calcium transients were completely eliminated by the specific AMPAR blocker CNQX. In contrast, the NMDAR blocker MK- 801 had no effect.

Conclusion: The spontaneous activity occurring under physiological conditions in cultured motor neurons and dorsal horn neurons is mediated via AMPA receptors and can therefore be used as a model for physiological AMPA receptor activation. Activity was synchronized, indicating the presence of pacemaker cells. Cytosolic calcium transients were triggered by AMPAR inward currents and spread throughout the whole neuron within ~100 ms, indicating a fast calcium-induced calcium release (CICR) from intracellular stores, e.g. the endoplasmic reticulum. The tight coupling between AMPAR activation and CICR may represent a molecular link between AMPAR input, energy metabolism and protein folding which are thought to be disturbed in the pathogenesis of ALS.

P74 ESTABLISHING A CELL MODEL TO STUDY DOWN-REGULATION OF THE GLUTAMATE TRANSPORTER EAAT2 BY SOD1^{G93A}

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Background: In amyotrophic lateral sclerosis, excessive activation of post-synaptic glutamate receptors may cause motor neuron degeneration through excitotoxicity. Loss of the glial glutamate transporter EAAT2 is a consistent observation in ALS patients and in transgenic rodent models, and EAAT2 down-regulation may contribute to motor neuron pathology. We have previously shown that SOD1^{G93A} causes down-regulation of EAAT2 in primary cultures of mouse astrocytes. A recent study by Sala et al (1) demonstrated that a neuronal-like SH-SY5Y cell line expressed EAAT2 and showed a down-regulation of glutamate transporter activity by SOD1^{G93A}, suggesting that this cell line might offer a convenient cellular model to examine pathophysiological EAAT2 regulation in ALS.

Objectives: To establish a cell model to elucidate the mechanisms by which SOD1^{G93A} causes a down-regulation of EAAT2. In this study we wished to determine whether it would be better to use the SH-SY5Y neuroblastoma cell line or to develop a new system for expression of recombinant EAAT2 in cell lines.

Methods: We characterized glutamate transporter expression in SH-SY5Y cells (passage 3–8) using RT-PCR with primers specific for each of the human glutamate transporters EAAT1–5 and Western blotting. Full length EAAT2 cDNA was derived by RT-PCR and cloned into the mammalian expression vector pcDNA3.1.

Results: RT-PCR showed that SH-SY5Y cells express mRNA for EAAT3 (EAAC1). Levels of mRNA for EAAT2 were negligible, even after 40 PCR cycles. EAAT1, 4 and 5 mRNAs were not present. Western blotting showed that EAAT2 migrates as a 66 kDa band and also as a higher band representing the multimeric form of the protein. However, SH-SY5Y cells express very low levels of EAAT-2 immunoreactive protein compared to rat frontal cortex, which was used as a positive control.

Conclusion: We conclude that, although EAAT2 is expressed by SHSY-5Y cells, the low levels of EAAT2 expression indicate that this cell line is not optimal for detailed biochemical studies of its down-regulation by $SOD1^{G93A}$.

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P75 EFFECT OF CEREBROSPINAL FLUID FROM MND PATIENTS ON SURVIVAL OF MOTOR NEURON CULTURES: IMPORTANT ROLE OF ASTROCYTES

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Background: Whether cerebrospinal fluid (CSF) contains soluble factors that contribute to the propagation of disease between motor neuron (MN) populations in motor neuron disease (MND) remains controversial. Multiple studies using different culture conditions have produced conflicting findings, suggesting cellular environment may be a factor in determining the effect of CSF exposure (1–3).

Objectives:

1. Investigate the effect of CSF from MND patients and control patients on the survival of MN-enriched cultures.
2. Compare the effects of MND/control CSF on MNs in isolation and in the presence of glial cells.

Methods: MNs were isolated from E14 Wistar rat embryos using established procedures and were grown either in mono-culture on laminin, or in co-culture on confluent astrocyte feeder layers. Cultures were exposed to CSF (either an artificial CSF (aCSF), or CSF from MND/control patients) at concentrations of either 20% or 50% for 24 h before fixation. Cultures were immunostained for neurofilaments and the number of MNs present in 20 random fields was counted in triplicate cultures.

Results: In MN-enriched cultures, neither MND nor control CSF was able to support MN survival comparable to normal culture conditions or aCSF. MN survival was significantly greater in the presence of MND CSF than control CSF ($p < 0.05$, Student's t- test). Conversely in co-cultures, control CSF supported survival equivalent to aCSF, whereas MND CSF significantly reduced survival ($p < 0.01$). Comparison of the effects of individual CSF samples in mono- and co-culture revealed an inverse correlation, where a CSF sample supporting greater survival in mono-culture was more likely to show toxicity to MNs in co-culture ($p = 0.001$).

Discussion and conclusions: The environment surrounding the MN appears to be pivotal in determining the effect of CSF on MN survival. Whilst control CSF is not sufficient to support survival when MNs are grown in isolation, trophic support from glial cells enables control CSF to support MN survival in co-cultures. The increased ability of MND CSF to support survival of isolated MNs compared to control CSF suggests the presence of additional trophic factors, although in co-culture toxic signalling, presumably from glia, overrides this to reduce survival.

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P76 *IN VITRO* PROPERTIES OF MICROGLIAL CELLS FROM G93A SOD1 TRANSGENIC MICE AND THEIR NON-TRANSGENIC LITTERMATES

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Background: Microglial cells are increasingly capturing scientific attention as possible generators or propagators of motor neuronal injury in ALS. The hypothesis of the non-cell autonomous damage to motor neurons proposes that the properties of neighbouring glial cells are altered by the presence of mutant SOD1 in SOD1-related ALS cases, and that these alterations are toxic to the motor neurons in the vicinity. Microglial cells, being the most immunocompetent cells of the CNS parenchyma, are more likely to secrete cytotoxic products, to which motor neurons may display a selective vulnerability.

Objectives: The objective of this study was to investigate whether the properties of microglial cells from the G93A SOD1 ALS mouse models compared to their non-transgenic littermates differ *in vitro*. The cellular properties investigated were: 1) TNF-α cytokine production; 2) cell number; 3) cell size; 4) cellular ramification; 5) F4/80 receptor expression; and 6) phagocytosis.

Methods: Primary microglial cells were obtained from motor regions of 1 to 2-day-old mouse pup cortices. The cells were cultured *in vitro* until they reached 30 days of age, then stimulated with bacterial lipopolysaccharide (LPS) for 24 h and incubated with labelled latex beads for 1 h. After stimulation, TNF-α production was measured using an ELISA kit. Morphological properties were analysed by immunocytochemistry and fluorescent microscopy.

Results: After LPS stimulation, both transgenic and non-transgenic microglial cells showed increases in TNF-α production, index of ramification, cell size and phagocytic ability. LPS caused a significant reduction in G93A SOD1 microglial cell numbers compared to that of the non-transgenic cells; fold changes were 0.912 for the non-transgenic and 0.478 for transgenic cells, $p = 0.038$. The levels of expression of the inflammatory receptor F4/80 with LPS stimulation were nearly doubled in the transgenic populations. We did not observe significant differences between the two cell types in TNF-α secretion (when corrected for cell number), cell size, index of ramification or phagocytic ability.

Discussion and conclusions: We describe here some of the *in vitro* properties of resting and activated wild-type and G93A SOD1 primary microglial cells. Our results show that at 30 days *in vitro*, wild- type or G93A SOD1 transgenic microglia do not differ in morphological characteristics investigated in this study. However, the sensitivity of transgenic microglial cells to environmental cues may be increased, as application of LPS significantly reduced the cell numbers in transgenic populations and showed a trend towards increased F4/80 expression. The mechanisms involved in the cell number reduction are currently under investigation.

P77 DIETARY FLAVONOIDS AT LOW CONCENTRATIONS ACTIVATE ERK, A PRO-SURVIVAL SIGNAL IN NEURONS

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Background: There are many studies that report neuroprotective actions of dietary-derived flavonoids. As well as their classical hydrogen-donating antioxidant activity, recent evidence suggests that flavonoids also exert modulatory effects on intracellular signalling pathways including ERK, a component of the pro-survival mitogen-activated protein kinase signalling cascade.

Objectives: We sought to characterize which of three key dietary flavonoids is likely to be the most potent neuroprotective agent by measuring their ability to regulate ERK phosphorylation. Representatives of different flavonoid classes were tested at concentrations relevant to those obtained through the diet.

Methods: Since flavonoid effects are dependent on cell type we used three culture systems: primary mouse cortical neurons, the neuron-like SH-SY5Y neuroblastoma cell line and human primary dermal fibroblasts (promocells). Cells were exposed for 15 min to the flavonoids over a concentration range of 10 nM to 10 μM. Flavonoids used were hesperetin (a flavonone), epicatechin (a flavonol) and quercetin (a flavonol). The cells were then lysed and Western blotting carried out, using a phospho-specific antibody to detect and quantify activated ERK1/2 (pERK).

Results: In neurons, hesperetin was the most efficacious flavonoid examined, potently increasing pERK at lower concentrations (peak effect at 100 nM). Hesperetin had similar effects in SH-SY5Y cells and in primary fibroblasts. In all cells examined, the concentration response to hesperetin was bell-shaped: at higher concentrations (greater than 1 μM), hesperetin caused a relative reduction in pERK. Epicatechin showed more modest increases in ERK phosphorylation compared to hesperetin. The effects of quercetin were highly dependent on cell type. In neurons, most concentrations of quercetin caused a net down-regulation in pERK compared to controls, whereas in SH-SY5Y cells, quercetin increased pERK levels, even at the highest concentration examined (10 μM).

Discussion and conclusions: Low concentrations of hesperetin, epicatechin and quercetin all caused increased pERK in each cell type, but with different concentration-response relationships that are cell type dependent. Our data predict that hesperetin, a flavonone which is particularly enriched in citrus fruit, is likely to be a neuroprotective flavonoid. The steps linking ERK phosphorylation and cytoprotection remain to be clearly elucidated. Data to examine the mechanisms underlying flavonoid-induced neuroprotection will be useful when considering dietary supplementation and modification as a potential therapeutic strategy in ALS.

P78 UNDERSTANDING THE MECHANISM OF IMPAIRED STRESS RESPONSES IN MOTOR NEURONS

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Background: Aggregation of abnormal protein occurs in familial and sporadic ALS, yet motor neurons fail

to mount a defensive heat shock response due to a high threshold for activating the transcription factor, Hsf1 (1). Lowering this threshold in a stress-sensitive manner has therapeutic potential in several neurodegenerative diseases. Binding of Hsf1 to heat shock elements (HSE) is not sufficient to initiate transcription of heat shock genes and various phosphorylation events are thought to control activation. Within the activation domain of Hsf1, phosphorylation of S303 by GSK3β and S307 by ERK1 decrease activation in cultured cells (2). Phosphorylation of S230 has been considered a requirement for induction of HSP expression and was induced experimentally by CaMKIIα (3).

Objective: This study tested the hypothesis that aberrant phosphorylation of key residues in the activation domain of Hsf1 is responsible for the high threshold for activating Hsf1.

Methods: Expression of stress-inducible Hsp70 was evaluated in motor neurons and glia of dissociated murine spinal cord cultures and in mouse embryonic fibroblasts in the presence and absence of heat shock (43°C for 1 h; 6 h recovery). Phosphorylation of key residues was modified by pre-treatment with the GSK3β inhibitor, lithium chloride (25 mM) or transfection with plasmid encoding a nuclear or cytoplasmic isoform of CaMKIIα or dominant negative ERK1.

Results: Lithium chloride failed to activate Hsf1 in heat shocked motor neurons, despite its effectiveness in non-neuronal cells. Motor neurons in spinal cord cultures lacked endogenous expression of a nuclear CaMKIIα isoform, which would be required to phosphorylate DNA-bound Hsf1, but over-expression of nuclear or cytoplasmic CaMKIIα did not result in expression of Hsp70 following heat shock.

Conclusions: Our studies so far indicate that failure of phosphorylation or dephosphorylation of serine residues in the regulatory domain of Hsf1 is not responsible for the impaired heat shock response in motor neurons. Induction of Hsp70 in motor neurons by the Hsp90-binding agents (steroid receptors, geldanamycin) suggests that interactions of Hsf1 with Hsp90 multi-chaperone complexes in the nucleus might underlie the high threshold for activation of heat shock gene expression in neurons.

Acknowledgement: This work was supported by CIHR/MDAC/ALS and ALSA.

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P79 UP-REGULATION OF HSP70 PREVENTS S-NITROSOGLUTATHIONE (GSNO)-INDUCED APOPTOSIS IN MOTOR NEURONAL CELLS EXPRESSING MUTANT CU/ZN SUPEROXIDE DISMUTASE (SOD1) VIA AN INHIBITION OF NF-κB

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Background and Objectives: Recent evidence suggests that nitric oxide (NO) toxicity is one of the primary mechanisms of motor neuronal degeneration. We previously reported that GSNO-mediated toxicity decreased viability of motor neuronal cells expressing mutant SOD1 (1). Hsp70 is a well-known heat shock protein that is cytoprotective against apoptosis in neurodegenerative disorders. In this study, we attempted to test the protective effect of Hsp70 on mutant SOD1 against NO-mediated toxicity.

Methods: Motor neuron neuroblastoma hybrid (VSC 4.1) cells were stably transfected with pMFG.puro vector containing Hsp70 cDNA and analyzed by staining or Western blotting using an Hsp70 antibody to investigate overexpression of Hsp70. To test the effect of Hsp70 on constitutively expressing mutant SOD1, VSC4.1 cells co-expressing Hsp70 and mutant SOD1 (A4V) were made. These cells were treated with GSNO and the viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. In order to investigate the features of cell death, caspase 3 activity was assayed in treated cells. To determine the target for the protective effect of Hsp70, the expression of the active p65 NF-κB subunit was measured by Western blotting.

Results: GSNO decreased the viability of cells expressing mutant SOD1 (A4V) in a dose-dependent manner. GSNO-induced mutant cell death was preceded by an increase of caspase 3 activation. Overexpression of Hsp70 in mutant cells blocked the GSNO-induced elevation of caspase 3 activity. In addition, up-regulation of Hsp70 prevented the expression and nuclear translocation of NF-κB produced by GSNO.

Conclusions: Our data showed an anti-apoptotic effect of Hsp70 against NO toxicity in motor neurons expressing mutant SOD1. This process seemed to involve a reduction of caspase 3 activation and levels of the active p65 NF-κB subunit. Our data suggest that Hsp70 may act as a suppressor of apoptosis and oxidative stress in motor neurons expressing FALS-linked mutant SOD1.

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P80 TETANUS TOXIN FRAGMENT C AND BCL-2 FUSION PROTEIN PREVENTS DEATH OF SERUM- AND NGF-WITHDRAWN PC12 CELLS

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Background: Tetanus toxin produced by *Clostridium tetani* is taken up by nerve endings at the neuromuscular junction and is retrogradely transported to the neuronal cell body. The C terminal region of the tetanus toxin (TTC) is in itself innocuous and has the same ability to be transported as the whole toxin. We explored whether the fragment of toxin can be applied to deliver a beneficial molecule to neuronal cells.

Objectives: Bcl-2, an anti-apoptotic protein, is reported to have prevented neuronal death in several experimental models *in vitro* as well as *in vivo*. We wished to fuse TTC with Bcl-2 in order to examine whether the fusion protein bound to neuron-like cells and whether it showed anti-apoptotic effect. Theoretically, if the fusion protein retains the characteristics of both molecules, it will exhibit an anti-apoptotic effect selectively in neurons.

Methods: The coding region of TTC was fused with Bcl-2 cDNA in the sequence Bcl-2 TTC or TTC Bcl-2. The constructs were introduced into prokaryotic expression vectors, which added a His tag signal sequence at the *N* terminus of the gene product. The fusion proteins were expressed in *Escherichia coli*. The recombinant proteins were purified by immobilized metal affinity chromatography. In binding experiments, PC12 cells were differentiated by NGF, then the fusion protein was added to the cells. Binding capability was observed immunohistochemically. Whether exposure of the fusion protein had an anti-apoptotic effect was examined by measuring its effect on the death of PC12 cells caused by withdrawal of growth factors (serum and NGF).

Results: TTC-Bcl2 and Bcl2-TTC fusion proteins were produced in *E. coli* and purified, although the purity of the protein was not sufficient. Among the purified proteins, Bcl2-TTC but not TTC-Bcl2 exhibited neuronal binding ability. Furthermore, Bcl2-TTC prevented neuronal death of PC12 cells induced by serum and NGF deprivation to some extent. To improve the yield and purity of the fusion protein, we deleted the N terminal portion of TTC from Bcl2-TTC, which we refer to as Bcl2-halfTTC (Bcl2-hTTC). Bcl2-hTTC was produced efficiently in *E. coli* and its purity was better than that of Bcl2-TTC. Bcl2-hTTC retained the same propensity as Bcl2-TTC with regard to neuronal binding and anti-apoptotic properties.

Discussion and conclusions: Bcl2-TTC and Bcl2-hTTC are anti-apoptotic *in vitro*. This intriguing nature of the fusion proteins could be a potent therapeutic agent in various neurodegenerative diseases, such as amyotrophic lateral sclerosis.

P81 TAUROURSODEOXYCHOLIC ACID (TUDCA), A BILE ACID, INHIBITS GSNO-INDUCED APOPTOSIS BY MODULATING REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN MOTOR NEURONAL CELLS EXPRESSING MUTANT CU/ZN SUPEROXIDE DISMUTASE (SOD1)

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Background: The dihydroxy bile acid ursodeoxycholic acid (UDCA) and its taurine conjugated derivative, tauroursodeoxycholic acid (TUDCA), are hydrophilic bile acids which are normally produced endogenously in humans at a very low level. Recent evidence has shown that UDCA and its conjugated derivatives play a role in modulating apoptosis in both hepatic and non-hepatic experimental models including neurodegenerative diseases.

Objectives: In this study, we examined whether TUDCA is neuroprotective in motor neuron-neuroblastoma hybrid cells (VSC4.1) expressing mutant SOD1 (A4V, G93A) against NO toxicity.

Methods: To test the effect of TUDCA on GSNO-mediated toxicity, TUDCA was pre-incubated in motor neuronal cells expressing wild-type (wt) or mutant (A4V, G93A) SOD1 and then treated with exogenous nitric oxide (S-nitrosoglutathione; GSNO) for 24 h. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To investigate the characteristics of neuronal cell death, cells treated with GSNO were stained with Hoechst 33342 and were assayed for caspase 3 activity. To examine the free radical formation, the concentration of peroxides was measured by flow cytometry using fluorescence emitted by DCFH oxidation. Bax expression and poly (ADP-ribose) polymerase (PARP) cleavage were also analyzed.

Results: GSNO-induced mutant cell death was significantly reduced by TUDCA, which decreased the number of Hoechst 33342-positive mutant cells and caspase 3 activity. Moreover, TUDCA prevented GSNO-increased ROS generation, Bax expression and PARP cleavage.

Discussion and conclusions: The results suggest that TUDCA may contribute to the protection of motor neurons from degeneration caused by SOD1 mutations through anti-apoptotic and anti-oxidant mechanisms. Therefore, it may provide a potentially useful treatment in patients with some forms of amyotrophic lateral sclerosis.

P82 THE ROLE OF ENDOPLASMIC RETICULUM-STRESS IN MOTOR NEURON DEGENERATION IN ALS MICE

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Background: Motor neuron degeneration in ALS has been proposed to occur by an active process of programmed cell death, and indeed many studies have shown the involvement of the mitochondrial apoptotic pathway. Less is known about the involvement of other organelles, such as the endoplasmic reticulum (ER), in motor neuron degeneration in ALS. ER stress occurs when misfolded proteins accumulate in the lumen of the ER. This initially results in the up-regulation of cytoprotective ER-resident chaperones, however if ER stress is severe and prolonged, cells eventually activate a caspase-dependent cell death programme. We have previously shown that the pro-apoptotic protein PUMA is necessary and sufficient for ER-stress-induced cell death in neurons.

Objectives: In this study we examined the expression of ER stress markers during disease progression in mutant SOD1 mice, and we also looked at the involvement of the BH3-only pro-apoptotic protein PUMA in mutant SOD1 motor neuron degeneration.

Methods: Using quantitative PCR and Western blotting, as well as immunohistochemistry, we examined the expression of ER stress markers and BH3-only proteins in tissue from mutant SOD1 mice during disease progression.

Results: Our studies have shown that up-regulation of ER-resident chaperones Grp78 and Grp94 is evident during early disease progression, when the up-regulation of other chaperones is becoming evident. Spinal cord sections immunostained with antibodies to Grp78 and Grp94 showed a large increase in expression in motor neurons. We also found an increase in the expression of the transcription factor CHOP during disease progression. The expression of the BH3-only protein PUMA increased progressively during disease progression, and interestingly was elevated during the early stage of disease progression, compared to wild-type littermates.

Conclusions: Our results suggest that ER-stress is actively involved in motor neuron degeneration in mutant SOD1 mice.

P83 A STUDY ON THE INTRA-MITOCHONDRIAL EFFECTS OF FALS-SOD1S: IS THERE A CORRELATION WITH THE BIOPHYSICAL CHARACTERISTICS OF THE PROTEINS ?

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Background: Degeneration of mitochondria represents a pathological hallmark in ALS. A significant fraction of FALS-associated mutant SOD1 is targeted to the mitochondria, where it forms aggregates containing proteins involved in the control of apoptosis. Proper folding of wild-type SOD1 depends upon the binding of metal ions and/or the redox state of specific cysteine residues. The disulphide status of SOD1 seems to play a pivotal role in the monomer-dimer equilibrium, stabilizing the quaternary structure of the protein.

Objectives: Data in cell or animal systems have been obtained only for a few mutant SOD1s, and no exhaustive analysis has been performed. In order to analyse the biochemical properties (propensity to form aggregates, monomer/dimer equilibrium, redox state of cysteine residues) of different SOD1 categories in the mitochondrial compartment of a neuronal environment, we have built a collection of inducible cell lines, derived from the mouse motor neuronal line NSC34, expressing a wide panel of SOD1 mutants.

Methods: The cDNAs coding for 12 FALS-SOD1s were expressed in NSC34-derived cell lines under the control of the inducible Tet-ON promoter, with or without a short C-terminal myc tail. For isolation of mitochondria, NSC34-derived cell lines were homogenized and subjected to sequential centrifugations at different speeds. The crude mitochondrial pellet was loaded onto a discontinuous gradient for further purification. Western blot detection of accessible FALS-SOD1 cysteines was performed on lysates from cytosolic and mitochondrial fractions. The samples were incubated in the presence of Mal-PEG for covalent modification of accessible cysteines. The addition of Mal-PEG to accessible cysteines increased the subunit mass of SOD1 by 5 kDa modification. Total and mitochondrial glutathione content was assayed in all NSC34-derived cell lines. Low molecular weight thiols were separated by HPLC.

Results: In a motor neuronal cell environment some FALS-SOD1s retain partial or full superoxide dismutase activity, whereas others do not. Mutants of the active site are present essentially in monomeric form. WT SOD1 is barely detectable in the mitochondrial compartment, while the fraction of FALS-SOD1s localized in the mitochondria

displays a decrease in cysteine accessibility. Moreover, most mutants do induce a decrease in the GSH/GSSG ratio inside mitochondria and this data parallels a marked decrease in complex I activity (NADH-ubiquinone oxidoreductase).

Conclusions: In agreement with the hypothesis that mitochondria are the site of primary damage in SOD1-linked FALS, the expression of FALS-SOD1 mutants induces a selective alteration in mitochondrial GSH/GSSG ratio and a failure in the respiratory activity of complex I. These alterations are related to the mitochondrial localization and cysteine oxidation state of FALS-SOD1s.

P84 ANALYSIS OF FALS- AND NON-FALS LINKED SOD1 GLYCINE-93 VARIANTS EXPRESSED IN MOTOR NEURONAL CELLS

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Background and objectives: Over 100 mutations in the SOD1 gene are implicated in familial forms of amyotrophic lateral sclerosis (FALS), yet predict proteins with diverse biochemical and structural properties despite a similar pathogenesis. Mutation of glycine residues occurs frequently in SOD1-linked FALS. In particular, codon 93 is point mutated to six possible variants in FALS, suggesting this glycine residue is critical for maintenance of native structure or function. Thus, glycine-93 may assume a local protein conformation forbidden to any other amino acid substitution. To test this hypothesis, murine motor neuron-like (NSC-34) cells expressing every possible SOD1 missense variant at position 93 were analysed for key biochemical parameters, including aggregation propensity, solubility, enzyme activity and pro-oxidant chemistry.

Methods: NSC-34 cells were transfected with pEGFP-N1 vectors containing human wild-type (wt), FALS-linked (G93A, G93C, G93D, G93R, G93S, G93V) or non-FALS linked (G93E, G93F, G93H, G93I, G93K, G93L, G93M, G93N, G93P, G93Q, G93R, G93S, G93T, G93V, G93W, G93Y) mutant SOD1 cDNAs. Cell transfectants were analysed using fluorescence microscopy, immunoblotting, zymography, dichlorofluorescein (DCF) oxidation and viability assays. SOD1 mutants were also visualized using molecular modelling and correlated with cell culture and biochemical data.

Results and discussion: The SOD1 glycine-93 mutants exhibited differential electrophoretic mobilities, activities, DCF oxidation and toxicity. However, cytoplasmic inclusion formation, impaired solubility and enhanced proteolysis were common to all 19 variants of SOD1. Thus, no clear relationship between aggregation and the substituted side chain properties (e.g. size, charge, polarity, hydrophobicity) was evident in mutants. These results suggest that preservation of glycine-93 is essential to maintain native SOD1 structure while preventing unfavourable self-association.

P85 IDENTIFICATION OF DIFFERENT ISOFORMS OF THE SOD1 PROTEIN IN A CELLULAR MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Familial amyotrophic lateral sclerosis (FALS) has been associated with mutations in the SOD1 gene encoding the Cu/Zn superoxide dismutase enzyme (SOD1). It has been hypothesized that the disease is not caused by a modification of the dismutase activity but by a gain of a new toxic function. The pathogenic mechanism is currently unknown, but studies made in other neurodegenerative diseases (1) suggest that the SOD1 protein is a major target of post-transcriptional modification.

Objectives: We have analysed, by proteomics tools, changes in expression and post-transcriptional modifications of the SOD1 protein in human neuroblastoma SH-SY5Y cells transfected with human wild-type and two different mutated SOD1 genes: H46R and G93A.

Methods: Protein extracts from each line were separated by 2D gel electrophoresis and protein spots were detected by the Coomassie and silver stains and analysed by PD-Quest software. SOD1 protein was identified by using a combination of mass spectrometry and Western blotting analysis. For the identification of the different isoforms of SOD1 protein, the gels of each line were transferred on to PVDF membrane. The membranes were probed with anti-human SOD1 antibody.

Results: SOD1 two-dimensional immunoblotting displayed four isoforms of the SOD1 protein with isoelectric points (pI) of 5.0, 5.7, 6.0 and 6.3 respectively. In particular, the SH-SY5Y line showed only three isoforms (pI 5.0, 5.7 and 6.3) while the isoform at pI = 5.7 is a characteristic of the transfected cell lines. The analysis of the two-dimensional gels showed an increase of the total quantity of the SOD1 isoforms in the transfected lines compared to the untransfected cells. Data for the expression of total SOD1 protein were confirmed by one-dimensional Western blotting. Two-dimensional gels also demonstrated that the isoforms show differences of expression.

Conclusions: The transfected cell lines express an isoform of the SOD1 protein not present in the untransfected cell line. Moreover, the four isoforms are differentially expressed in the WT, G93A and H46R lines. These data indicate that the overexpression of the SOD1 protein in the transfected lines induces post-transcriptional modification of the protein as suggested by the appearance of a new isoform.

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P86 ALS2/ALSIN REGULATES RAC-PAK SIGNALLING AND NEURITE OUTGROWTH

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Background: Mutations in ALS2 are causative for some recessive familial forms of ALS. The structure of ALS2 predicts that it functions as a guanine nucleotide exchange factor (GEF). GEFs regulate the activity of members of the Ras superfamily of GTPases. ALS2 contains three putative GEF domains, the central region containing Dbl and pleckstrin homology domains that are found in GEFs for Rho family members. The recessive nature and types of mutations in affected families strongly suggest that a loss of ALS2 function is the primary cause of disease. As such, a proper understanding of the molecular mechanisms by which mutant ALS2 induces motor neuron disease requires insight into its function.

Objectives: The objectives were to gain insight into ALS2 GEF function.

Methods: The subcellular distribution of ALS2 in neurons was examined by immunocytochemical analyses. The effects of ALS2 on the activities of Rho family members and PAK1, and on neurite outgrowth were all investigated using cellular assays.

Results: Activity assays revealed that ALS2 stimulated the activity of Rac but not Rho or Cdc42. Rac regulates the activity of PAK family kinases and ALS2 also stimulated PAK1 activity. Mutation of the ALS2 Rac GEF domain abrogated these effects. During development, Rac-PAK signalling functions to regulate actin dynamics in the growth cone so as to control neurite outgrowth. ALS2 localized within growth cones of neurons and expression of ALS2 significantly promoted neurite outgrowth by approximately 1.5-fold. Disruption of the ALS2 Rac GEF domain and expression of dominant negative Rac but not dominant Rab5 abrogated the stimulatory effect of ALS2 on neurite outgrowth.

Discussion: We demonstrate that ALS2 is present within neuronal growth cones and that it functions as a GEF for Rac so as to stimulate Rac-Pak signalling and neurite outgrowth. Mutations in ALS2 may therefore induce disease by compromising proper development of motor neurons, making them more susceptible to later toxic insults.

P87 AMYLOID PRECURSOR PROTEIN OVEREXPRESSION IN A CELLULAR MODEL OF AMYOTROPHIC LATERAL SCLEROSIS.

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Background: Some studies indicate that amyloid precursor protein (APP), the transmembrane precursor of β-amyloid peptide, increases as a consequence of central nervous system insults (i.e. cholinergic lesions, hypoxic-ischaemic injury, traumatic brain injury) at an early stage after damage. Recently, it was also observed that APP is increased in the anterior horn motor neurons of ALS patients. Also, mutations in SOD1 associated with amyotrophic lateral sclerosis (ALS) confer enhanced pro-oxidative enzyme activities. Thus, mutant SOD1-mediated ALS conforms to classic neurodegenerative diseases such as Alzheimer's disease (AD), by a common, not well known, neurodegenerative pathway, which causes self-aggregating and neurotoxic proteins to accumulate in the central nervous system.

Objectives: The present study examines the potential link between pro-oxidative G93A SOD1 and the expression of some components influencing amyloidogenesis. In fact, a causal relationship between oxidative stress and AD is well known. With this aim, we examined whether the expression of some Alzheimer's genes was modulated in cortical cell cultures of transgenic ALS mice.

Methods: Three different cultures were prepared, murine, expressing human SOD1 (hSOD1) and G93A mutated hSOD1 cells. A real time PCR method was used to analyse the APP, PSEN1 and PSEN2 expression. Total RNA from each sample was used to generate cDNA by reverse-transcriptase and random primers. For quantitative PCR, primers and probe mixtures specific for each gene were used. The relative quantification was performed using the comparative C_T method and 18s as an endogenous reference.

Results: Our data show a significant overexpression of APP in cortical G93A cultures compared to hSOD1 cultures, which were used as controls (1.8 ± 0.1 vs. 1 ± 0.1; $p < 0.05$). An increase of PSEN1 and PSEN2 mRNA levels was also evident, but without any statistical significance.

Discussion and conclusions: Our results indicate a different transcriptional response of APP with respect to presenilins induced by the mutated SOD. Increased APP could correspond to the brain β-amyloid accumulation in mutant SOD1 according to Turner et al. These findings indicate that G93A SOD1 in the cortical cultures may

serve to up-regulate APP synthesis and indirectly contribute to β-amyloid deposition by means of higher substrate availability for the proteolytic enzymes of the amyloidogenic pathway.

P88 GENE EXPRESSION PROFILE OF SPINAL MOTOR NEURONS IN SOD1 G93A MOUSE MODEL

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Background: Amyotrophic lateral sclerosis is one of the most common adult onset neurodegenerative diseases, characterized by progressive and selective degeneration of the upper and lower motor neurons in the motor cortex, brain stem and spinal cord. The causative mechanism of this cell-specific loss is still unknown.

Objective: The aim of this study is to investigate the changes in gene expression profiles of degenerating spinal motor neurons isolated from human SOD1 G93A and SOD1 WT mice and non- transgenic littermates at 60, 90 and 120 days in order to identify which pathways are involved in the development of the neurodegenerative process.

Methods: Approximately 1000 motor neurons were isolated from lumbar spinal cord. RNA was extracted using Picopure kit (Arcturus), amplified using the RiboAmp Amplification kit (Arcturus) and labelled using the BioArray High Yield RNA Transcript Labelling Kit (Enzo). 10 μg cRNA was applied to the Affy MOE430A GeneChip, and data analysis was performed using ArrayAssist System (Iobion).

Results: Initial studies comparing motor neurons from 120-day-old SOD1 G93A, SOD1 WT and their respective non-transgenic littermate mice show a significant change in expression of 241 genes of the 14,000 arrayed, when applying criteria of changes of two-fold or higher. One hundred and fifty of the genes examined are down-regulated, and 91 are up-regulated. The genes have been categorized according to their molecular function, and include genes involved in the transcription process, antioxidant and stress responses, apoptosis, and protein degradation. Consistent with results obtained by the analysis of the NSC34 cell line transfected with vector expressing human G93A SOD1 (1), the transgenic mice also show a marked degree of transcriptional repression. Interestingly, genes involved in antioxidant activity and stress response are significantly decreased, underlining the important role that oxidative stress plays in ALS. No significant differences have been found comparing SOD1 WT mice with their littermates.

Discussion and conclusions: We aim to present gene expression changes identified in earlier stages of disease, in order to find out which cellular functions are initially impaired and to determine which processes are involved in motor neuronal degeneration. Microarray technology combined with laser-capture microdissection is the most appropriate approach to understanding the motor neuron-specific expression profile related to the degeneration process occurring in ALS, as demonstrated by Jiang et al. (2). It enables the isolation of single cell types allowing us to overcome bias of motor neuron loss, reactive astrogliosis and other cellular reactions that interfere with the analysis of motor neuron-specific transcripts.

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P89 DEVELOPMENT OF A THREE-DIMENSIONAL TISSUE-ENGINEERED *IN VITRO* MODEL TO STUDY MOTOR NEURON DISEASES

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Background: Motor neuron disorders such as amyotrophic lateral sclerosis are very complex diseases and their development and progression involve many cellular processes and many cell types. Current *in vitro* models include neuronal cell lines, primary cell cultures and three-dimensional (3-D) tissue slices. Primary cell cultures usually contain mixed cell types and dividing support cells rapidly overcome neurons, while tissue slices do not allow the addition or removal of specific cells. Thus, a good *in vitro* model for the study of motor neuron disorders in a 3-D physiological environment would fill the gap between elementary models of cell culture and complex models of transgenic mice.

Objectives: Taking advantage of our background in tissue engineering (1,2), our aim was to develop a highly physiological 3-D model that could facilitate the study of motor neuron diseases *in vitro*. Specific objectives were to purify motor neurons and to incorporate them into a remodelled 3-D biomaterial (sponge) containing an extracellular matrix and environment that could allow the culture and neurite extension of motor neurons.

Methods: We seeded mouse fibroblasts alone or with Schwann cells onto collagen and chitosan sponges and cultured them in the presence of ascorbic acid. We isolated motor neurons from embryonic mouse spinal cords and purity was determined by staining of Hb9 followed by FACS analysis. Motor neurons were added freshly to the sponges that were further cultured with or without neurotrophic factors (BDNF, GDNF, CNTF and NT-3). Histological analysis was performed and neurite outgrowth was assessed by immunofluorescence staining of neurofilaments.

Results: The technique yielded more than 90% of Hb9-positive motor neurons. Culture of the sponges seeded with mouse fibroblasts or mouse fibroblasts and Schwann cells allowed a good production of extracellular matrix. This suited the purified motor neurons well (which are usually difficult to grow in culture), as they survived in the models and extended neurites in the sponges. However, the addition of neurotrophic factors was required in order to induce a deeper extension of motor neuron neurites into the models.

Discussion and conclusions: We developed a 3-D tissue-engineered *in vitro* model that mimics the physiological environment of motor neurons and allows their culture and neurite extension. We will further complete the model by adding and co-culturing support cells that are possibly implicated in the pathogenesis of motor neuron diseases (astrocytes, microglial cells, endothelial cells). Thus, this model represents a powerful tool for the study of motor neuron disorders.

Acknowledgements: This study was supported by the Muscular Dystrophy Association and by the Fonds de la Recherche en Santé du Québec.

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P90 BONE MARROW STROMAL CELLS PROMOTE PROLIFERATION OF POTENTIAL NEURAL STEM CELLS IN ORGANOTYPIC SPINAL CORD SLICE CULTURES

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Background: Recent reports have suggested that transplantation of bone marrow stromal cells (BMSCs) could rescue neurons in ischemic brains and in injured spinal cords of rodent models. They also promoted proliferation of stem cells in the subventricular zone (SVZ). However, details of the mechanism remain unclear. Clinical trials are now underway among human ALS patients, although a basic understanding has not yet been established.

Objectives: To investigate the effect of BMSCs on spinal cord, we established slice cultures from rat spinal cord that were maintained with BMSCs in the same wells (bi-culture).

Methods: BMSCs were obtained from adult Sprague-Dawley rats and cultured in plastic flasks. The spinal cords were isolated from the same strain on postnatal day 6, and cut into 400 μm-thick transverse slices. The slices were cultured on membranes inserted in six-well-plates on the bottom of which BMSCs had been seeded in advance.

Results: The slice cultures without BMSCs increased their thickness and showed marked gliosis especially in the margin of slices from 10 days *in vitro*. However, cultures with BMSCs did not become thick. The protein content of bi-cultured slices was half that of conventional cultures. Immunohistochemical analysis of bi-cultures by GFAP staining showed less glial response. Furthermore, nestin-positive cells proliferated around the margin of slices. They had taken up BrdU soon after slice-preparation.

Discussion and conclusion: The results of this study suggest that BMSCs can promote the proliferation of nestin-positive cells instead of gliosis in spinal cords. Those newly divided, nestin-positive cells could be potential neural stem cells, though we have yet to confirm whether they can differentiate into neurons in the next step. One of the practical problems for regenerative therapy is the source of stem cells. BMSCs could be a useful tool to solve this problem.

P91 IDENTIFICATION OF COMPOUNDS CAPABLE OF REGENERATING MOTOR AXONS BY SMALL MOLECULE SCREENING PROGRAM

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Background: ALS is characterized by the loss of specific motor neuron pools and the associated degeneration of motor axons. A large number of studies have focused on finding ways to prevent motor neuron cell death. Finding treatments able to rescue motor neuron soma would be very promising. However, to assure the complete recovery of motor function, the discovery of compounds capable of regenerating motor nerves is critical.

Objectives: The aim of this study was to identify molecules capable of inducing motor axon growth.

Methods: To do this we treated post-natal rat spinal cord organotypic cultures with drugs from a library of 1040 Food and Drug Administration-approved drugs and nutritionals. Tissue cultures were incubated for 7 and 14 days with 10 μM of each drug (n = 10 cultures/drug), then collected and stained for neurofilament-bearing axons with SMI-32. All cultures were blindly rated for axon outgrowth by qualitative and quantitative scales. Glial-derived neurotrophic factor (GDNF)-treated cultures served as positive control, while DMSO-treated cultures served as a vehicle control. All positive hits were re-evaluated by treating the organotypic cultures with different doses of drugs.

Results and conclusions: Multiple compounds were found to induce motor neuron axon growth *in vitro*. To validate the potential of these agents *in vivo*, we will assess them in various paradigms of acute axonal injury such as facial nerve axotomy and sciatic nerve crush.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 115–126

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THEME 5 IMPROVING DIAGNOSIS AND PROGNOSIS IN MOTOR NEURON DISEASES

P92 DETECTION OF ALS BY QUANTITATIVE REAL TIME RT-PCR OF PERIPHERAL BLOOD CELLS

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Background: Recent work by our group has established that blood macrophages from individuals with amyotrophic lateral sclerosis (ALS) have significant changes in their transcriptional program compared to healthy individuals. This raises the possibility that diagnosis of ALS can be assisted by molecular analysis of blood specimens.

Objective: To evaluate the utility of a quantitative RT-PCR assay of peripheral blood cells for the classification of samples as derived from ALS patients or controls.

Methods: Blood samples were obtained from patients with ALS and controls after obtaining informed consent and mononuclear cells were isolated by Percoll gradient centrifugation. The cells were cultured overnight at 37°C under non-adherent conditions, collected, lysed, and total RNA prepared. Aliquots of the RNA preparations were then RT-PCR amplified with primer pairs specific for five human genes using a Light-Cycler. The signals obtained were normalized against the signal obtained with primers for β-actin. A classification algorithm was built according to published methods (1) using signals obtained from 13 randomly selected ALS patients and 11 healthy controls. The algorithm was then tested using additional samples derived from ALS patients (n=18), healthy controls (n=9), and individuals with macular degeneration (MDgn) (MDgn, n=16). Operators of the Light-Cycler were blinded with respect to the classification algorithm. Individuals with MDgn were evaluated as controls because they also have activated macrophages in their blood.

Results: In the training set 12 of 13 samples from ALS patients and 0 of 11 control samples were classified as ALS-like. In the test samples 17 of 18 ALS samples, 0 of 9 control samples, and 0 of 16 MDgn samples were classified as ALS-like. All eight samples obtained from ALS patients within six months of diagnosis were classified as ALS-like and there was no obvious trend towards higher or lower scores with advanced disease; nor was use of riluzole or other medications by ALS patients associated with significant changes in classification scores.

Conclusions: A classification system based on the transcription level of five genes expressed in peripheral blood macrophages of ALS patients exhibited ~95% sensitivity (29 of 31 samples/24 of 25 patients) and 100% specificity (0 of 36 controls) at identifying ALS patients. Thus diagnostics based on alterations in gene expression seen in peripheral blood macrophages hold great promise for the early detection of ALS. Ongoing studies are focused on evaluating the classification index in patients with other neurological diseases.

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P93 CIRCULATING ANTIBODIES TO CYSTEINYL CATECHOLAMINES IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background: Recent studies, so far not related to motor neurons, have shown *in vivo* formation of cysteinyl-catecholamine (Cyst-CA) compounds, which may have a neurotoxic activity. These compounds result from the oxidation of catecholamines followed by the nucleophilic reaction with thiol residues such as cysteine.

Objectives: Using a routine ELISA methodology, circulating antibodies (IgG, IgM and IgA) to Cyst-CA were searched in ALS patients' sera.

Methods: All sera were obtained with the patients' informed consent. Two groups of patients were studied: 1) 27 ALS patients (40–80 years old) affected at various sites of onset, used for preliminary screening; 2) another, larger group of 49 patients (40–80 years old) classified according to onset of the disease, including sera from 17 patients with a bulbar onset (10 males and 7 females), sera from 14 patients with an upper onset (10 males and 4 females), and sera from 18 patients with a lower onset (15 males and 3 females). These groups were being treated with riluzole®. The control and comparative populations in our studies were 1) healthy controls (n=19, 37–70 years old); 2) patients with Parkinson's disease (n=23, 40–80 years old) and patients with multiple sclerosis (n=25, 40–80 years old). We synthesized Cyst-CA compounds, mimicking their endogenous formation

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045032

by linking N-acetyl-cysteine (NAC) with catecholamines: dopamine (DA), L-3,4-dihydroxyphenylalanine (L-DOPA), adrenaline (A), noradrenaline (NA), or homovanillic acid (HVA). The different groups of patients were statistically compared to the corresponding control groups using the Mann-Whitney *U*- test.

Results: In a first series of experiments, the antibody titres were compared to those of controls and patients with other neurodegenerative diseases. Significant antibody levels were found for Cyst-CA. The G and A isotypes were found in contrast to the M isotype. A second series of experiments showed that A and G titres were elevated, depending on the type of Cyst-CA and the onset of the disease. IgG for NAC- L-DOPA were present in cases of bulbar and upper onsets. IgA for NAC-HVA, NAC-A, and NAC-DA were found in lower onset.

Conclusions: For the first time, we have found circulating antibodies, in the sera of ALS patients, directed against Cyst-CA compounds of IgG and IgA isotypes. No IgM antibodies were found.

These results indirectly show that 1) the oxidation of CA and the formation of Cyst-CA may be involved in ALS; 2) these radical processes have different targets depending on the onset of the disease. These findings show that the extent, as well as the variety of radical processes in ALS is larger than expected.

P94 PROTEOMICS ANALYSIS OF MOTOR NEURON DISEASE PATIENTS' CEREBROSPINAL FLUID

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MND is a severe fatal syndrome characterized by the progressive neurodegeneration of motor neurons. Familial and sporadic forms of the disease have indistinguishable clinical and histopathological features suggesting common molecular pathogenic mechanisms. Several hypotheses have been formulated; however, the disease mechanisms are largely unknown. One of the limitations in MND is the difficulty of diagnosis in the early stages of the disease, due to the absence of specific markers.

For this reason and to investigate pathological mechanisms we analysed the patients' CSF; this fluid surrounds the brain and spinal cord and it may be expected to reflect some changes occurring in the central nervous system, probably involved with the progression of the disease.

This analysis has been performed using the proteomic approach, a technique that allows the separation of a large number of proteins. The term refers to the study of the complete set of proteins that are expressed by the genome of a cell in a defined physiological or pathological condition. This approach includes the generation of two-dimensional gel electrophoresis (2DE) stained maps that resolve proteins on the basis of the isoelectric point and relative mass. It is combined with protein identification by mass spectrometry (MS) and comparisons with reference to 2DE maps of many tissues, fluids, and cell lines in different conditions. By comparing proteins coming from healthy donor versus MND patients we aim to identify differentially expressed proteins produced by pathological mechanisms. Initial experiments show a protein isoform expression reduced in MND patient cerebrospinal fluid versus healthy controls.

P95 CEREBROSPINAL FLUID OXIDIZED PROTEINS AND ANTIOXIDANT CONTENT ARE DIFFERENTIALLY MODIFIED IN THE DIFFERENT FORMS OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a neurological disorder characterized by a progressive upper and lower motor neuron loss, usually beginning in late adulthood or in senescence and with a mean survival time not longer than three years from the diagnosis. ALS causes are unknown, but accumulating evidence including the occurrence of mutations in superoxide dismutase 1 (SOD1) gene in 20% of familial cases, indicate that oxidative stress is involved in the pathogenesis of this disease. In fact, an imbalance in the equilibrium between oxidative stress and endogenous antioxidant capacity can be responsible of damage to proteins, lipids, and DNA and can contribute to motor neuron degeneration.

Objectives: The aim of this study was to test the hypothesis that oxidative stress contributes to the pathogenesis of ALS. To do this we chose to assess 1) a generic marker of oxidative stress, i.e. the overall content of antioxidants in the cerebrospinal fluid (CSF), determined by its ferric reducing ability (FRA) and 2) a specific index of oxidative damage to proteins by reactive oxygen species, i.e. the amount of advanced oxidation protein products (AOPP).

Methods: Samples of CSF were obtained from 49 consecutive ALS patients (10 presenting with the bulbar form, 17 with the pseudopolyneuropathic form, 22 with the classical form of the disease), mean age $\pm$SD, 62.73 ± 10.82 years and eight control subjects, mean age 41.25 ± 14.64 years, with neurological diseases different from ALS and absence of CSF biochemical anomalies at standard laboratory analysis.

Results: AOPP results were higher in ALS patients (mean $\pm$SE, 13.2 ± 1.7) compared to control group (0.75 ± 0.55), $p=0.0039$. However, when subdividing patients, those with the bulbar form had AOPP levels significantly lower ($p<0.001$) than patients with the classical or the pseudopolyneuropathic form (1.1 ± 0.59, 15.35 ± 2.47, and 18.02 ± 2.57, respectively). FRA was

found to be lower in patients with respect to controls (48.8 ± 2.2 and 61.3 ± 3.2, respectively, $p < 0.03$), but no difference was found between the different forms of ALS.

Conclusions: ALS has been found to be associated with a decrease of CSF antioxidant ability as assessed by FRA, and increase of oxidative damaged to proteins, as assessed by AOPP. The fact that AOPP levels, but not FRA, are differently affected in the three forms of ALS suggests that although oxidative stress is involved in the pathogenesis of ALS, the precise mechanisms and nature of the oxidative challenge to CNS might be specific for each form.

P96 IDENTIFICATION OF CEREBROSPINAL FLUID POTENTIAL BIOMARKERS OF AMYOTROPHIC LATERAL SCLEROSIS

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Background: High-throughput protein expression analysis provides rapid and comprehensive screening for proteins in biological fluids and therefore is potentially useful for the identification of novel protein biomarkers. Amyotrophic lateral sclerosis (ALS) is a disease with a diagnosis based entirely on clinical features. Identification of a true biomarker for ALS is important for diagnosis and may provide insight into the pathogenesis of this disease.

Objective: To determine if there is a specific protein profile in the cerebrospinal fluid (CSF) that separates patients with ALS from those with pure motor and peripheral neuropathy (PN) and normal healthy control subjects.

Methods: CSF from normal healthy controls, ALS and neurological control cases followed at Mount Sinai School of Medicine (MSSM), Massachusetts General Hospital (MGH) and University of Washington (UW) were analyzed by surface enhanced laser desorption/ionization (SELDI)-time of flight (TOF) mass spectrometry (SELDI)-MS ProteinChip technology. Sensitivity and specificity of each marker were calculated with receiver operating characteristic (ROC) curve methodology.

Results: Samples were divided into discovery and validation protein profile analysis studies. In discovery studies, we identified two protein species (6.7 kDa and 13.4 kDa) of significantly lower concentration in the CSF of ALS patients ($n=36$) compared to normal control ($n=21$) cases. Using the ROC analysis, we found that a combination of the 6.7 and the 13.4 kDa protein species (the '2-protein' model) predicted ALS more accurately than either protein species alone (92% accuracy, 91% sensitivity and 92% specificity in correctly identifying all ALS and non-ALS patients). Independent validation studies using separate cohorts of ALS ($n=14$), normal healthy controls ($n=25$) and patients with pure motor and sensorimotor peripheral neuropathy (PN) ($n=7$) confirmed the ability of the 6.7 kDa and 13.4 kDa CSF protein species to separate patients with ALS from other diseases.

Conclusions: This SELDI-MS study suggests that additional application of '2-protein' biomarker model to current diagnostic criteria may provide an objective biomarker pattern to help identify patients with ALS.

Acknowledgement: This study was supported by MDA and Department of Veterans Affairs, ALS grant to GMP.

P97 THE SKELETAL MUSCLE TRANSCRIPTOME IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: The mechanism underlying ALS pathogenesis is still unclear but growing evidence suggests that initial alterations in skeletal muscle, preceding the onset of disease symptoms, may contribute to the disease process.

Objectives: We compared the muscle transcriptome of ALS-related SOD1(G86R) mice with that of sciatic nerve-axotomized mice. We also analyzed gene expression in skeletal muscle biopsies obtained from ALS patients and control subjects as a means to identify new potential markers of ALS severity.

Methods: We used a high-density oligonucleotide microarray and applied advanced bio-informatics protocols to provide a high throughput and accurate analysis of gene expression on a large scale.

Results: An unsupervised two-dimensional clustering algorithm identified genes that are highly regulated in SOD1 (G86R) mice. While some of these genes represented the characteristic denervation process occurring in ALS, others were specifically regulated under the pathological condition but not after experimental denervation. In man, the statistical analysis revealed two groups of genes that categorized patients with defined

characteristics. The first group included genes systematically repressed in all ALS patients, and the second one contained genes up-regulated in correlation with the severity of motor impairment.

Conclusions: Skeletal muscle provides an additional source of information for the comprehension of the pathological mechanisms acting in ALS. The systematic approach at the gene level presented herein will serve to identify new targets for therapeutic intervention and diagnosis.

P98 PHASE 2B RANDOMIZED DOSE-RANGING CLINICAL TRIAL OF TAMOXIFEN, A SELECTIVE ESTROGEN RECEPTOR MODULATOR [SERM], IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): SENSITIVITY ANALYSES OF DISCORDANCE BETWEEN SURVIVAL AND FUNCTIONAL OUTCOMES WITH LONG-TERM FOLLOW-UP

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Background: ALS patients were assigned to blocks ($n=5$) matched by age, sex, ALS onset site and baseline ALS Functional Rating Scale-total score (ALS-FRSt). Within each block, patients were randomized to one of five tamoxifen doses. Planned as a 12-month clinical trial, it was extended to 24 months when there was no statistically significant change in endpoints (rate of computerized isometric muscle strength (CIMS) loss, vital capacity (VC) loss, ALS-FRSt decrease) across the tamoxifen dose cohorts at 12 months.

Methods: Evaluations were at three- month intervals over 24 months. Follow-up provided survival and event history data for achieving milestones in CIMS arm and CIMS leg, VC and ALS-FRSt. Endpoints were analyzed by intention-to-treat allocation employing the Kaplan-Meier method with log-rank test and Cox proportional hazards modelling.

Results: ALS patients entered (34 males 26 females) had a mean age of 51 years, and ALS-FRSt of 24 with no significant difference in these parameters among the five dose groups at baseline. Protocol analysis of the difference in survival for the two lower dose cohorts together versus the three upper cohorts together was significant ($p<0.04$ two-sided safety p value). Cox proportional hazard analyses supported a drug dose effect. Prolongation of 80% survival benefit was above 200 days in the 20 mg, 30 mg, and 40 mg daily tamoxifen treatment cohorts. Survival in the 10 mg weekly-10 mg daily cohorts was identical to that recorded for the ALS patients randomized to placebo in the Dutch ALS Creatine Study (1). No significant dose-related adverse events occurred throughout the clinical trial. Safety analysis in a single-site phase 2B dose-ranging clinical trial of tamoxifen in ALS patients indicates a survival benefit of tamoxifen treatment with 20–30–40 mg daily compared with 10 mg weekly–10 mg daily. Sensitivity analyses do not support baseline age, sex, site of onset, duration of ALS, baseline ALS-FRSt, baseline VC, or co-morbidities as explanatory for this observed survival effect. Slower decrease in VC of the 20 mg, 30 mg, and 40 mg daily tamoxifen treatment cohorts in the second 12–24 month epoch might explain the survival differential but this was not statistically significant.

Conclusions: This survival benefit occurred in riluzole-treated ALS patients and was as large or larger than the improved survival in riluzole-treated compared with placebo-treated ALS patients. This observation needs to be replicated in an appropriately statistically powered clinical trial to establish whether tamoxifen should be considered as an adjuvant therapy with riluzole in the treatment of ALS.

Acknowledgement: The study was supported by the Muscular Dystrophy Assocation, ALS Division, and National Institute of General Medical Sciences General Clinical Research Center. Tamoxifen was provided by Astra-Zeneca Pharmaceuticals.

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P99 A HISTORICAL DATABASE CONTROLLED PHASE II STUDY OF INTERFERON BETA 1A TREATMENT IN ALS

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Background: In ALS, the presence of innate and adaptive immune reactivity supports a potential role for pro-inflammatory mechanisms in motor neuron injury. Interferon beta 1a (IFNβ1a), is an approved immunomodulatory drug for multiple sclerosis. Although the mechanisms of action are not completely defined, IFNβ1a clearly modulates both innate and adaptive immune responses, and may have beneficial effects in the treatment of ALS

Objective: To investigate the effect of IFNβ1a upon disease progression in ALS in a Phase II contemporary database-controlled study.

Methods: A 12-month Phase II single-center, historical database controlled study of 60μg IFNβ1a and disease progression in sporadic ALS was used. Historical controls were derived from a contemporary database of the MDA/ALS clinic (Houston, TX), based upon study criteria. The

primary outcome was post-baseline slope of total Appel ALS score (AALS) to end of treatment. Secondary outcome measures included post-baseline slope of AALS total score at 3, 6, 9, and 12 months; post-baseline slope of the FVC, time to 20 point progression of AALS score, time to a FVC 50%, and survival. Statistics were based on an intent-to-treat principle using Student's *t*-test or Mann-Whitney test for group comparisons. Log rank analysis was used to compare times to clinical endpoints.

Results: Twenty-nine patients were enrolled from July 2001 to July 2003. A contemporary, matched, database control group ($n=43$) was selected according to inclusion/exclusion criteria. Fourteen patients completed the treatment period, with 21.4% discontinuing drug due to adverse events. The INFβ1a treated group showed a slower disease progression as measured by rate of change of AALS total score over 12 months compared to the database control population (3.76 ± 3.56 pts/months vs. 4.94 ± 3.42 pts/month; $p=0.042$). Slower progression was also observed at three and six months with a trend toward significance at 9 months in the IFNβ1a treated group. No significant difference was observed in the time to 20 point progression in AALS total score from baseline between groups ($p=0.3$), although the IFNβ1a treated group did show a delay in time to 20 point drop in FVC (logrank $p=0.027$) and to 50% FVC from baseline (logrank $p=0.018$) compared to the database population. Prolonged survival was associated with IFNβ1a therapy independent of pre-slope and baseline AALS which were also found to be covariates of survival.

Conclusions: IFNβ1a treatment is associated with slowing of disease progression and prolongation of survival in an ALS patient population matched to a contemporary database-controlled population. Thus, IFNβ1a appears to warrant further study as a potential therapy in ALS with a Phase III randomized placebo-controlled trial.

P100 MULTI-DRUG 'COCKTAIL' THERAPY IN ALS: A FEASIBILITY AND SAFETY STUDY

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Background: The use of multiple medications, supplements, and homeopathic compounds in the treatment of ALS is almost universal. Often patients and physicians devise their own cocktail of agents which are untested in controlled trials. The design and conduct of such trials are challenging and the feasibility of testing multi-drug therapy needs to be better defined prior to onset of a clinical trial accessing efficacy.

Objectives: To assess the safety and feasibility of administering an eight-drug combination trial in patients with ALS. Compliance was tested with two methods of drug administration. Safety issues (adverse events) were evaluated with a flow chart of anticipated side-effects and a predetermined protocol of dose manipulation.

Methods: Eleven patients were enrolled in the open label multi-drug study. Inclusion criteria included probable or definite ALS, forced vital capacity greater than 50%, disease duration of less than 5 years, and at least 5 or 10 upper extremity muscle groups with MRC grade of 4 or better. Patients were given medication instructions, photographs of the medication regimen for each time point (morning, lunch, dinner, evening), and diaries. Five patients received their medications pre-dispensed in weekly medication dispensers and six patients received the medications in individual pill bottles. Patients returned at months 1, 2, 3, 5, 7, and 9 for follow-up evaluation. Potential side-effects were identified at the onset based on our experience with the components of the cocktail. Stepwise procedures were established for dose reductions or cocktail modifications to accommodate adverse events.

Results: Patients were able to maintain the medication regimen and tolerated the large number of medications well. Compliance was greater in the group of patients that received the medication pre-dispensed in weekly medication dispensers (99% compliance vs. 89% compliance). Four patients in the second group (pill bottles) began using pill dispenser or similar methods on their own. Patients were encouraged to keep a medication diary, recording any missed doses as well as any adverse events that occurred. Compliance with the medication diary was 75%. Our method of dose modification was successfully implemented for most adverse events. Individual components of the cocktail were, systematically, either reduced or stopped with appropriate resolution of the adverse event.

Conclusion: Patients were able to maintain compliance with the large number of medications and daily regimen, though providing patients with daily medication dispensers did improve compliance. Overall, adverse events occurred less often and with less severity than expected. The method of responding to adverse events with predetermined modification to the cocktail was effective and easily administered. The feasibility of designing and implementing a multi-drug therapy is supported by these data. Subsequent study in an efficacy trial is warranted.

P101 FINDING AN INDIVIDUALIZED METHOD FOR MEASURING PROGRESSION IN ALS CLINICAL TRIALS

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Background: Clinical trials of ALS therapies often compare the slopes of an outcome measure between treatment groups to measure treatment efficacy. The variability of ALS presentations may cause some muscles to deteriorate initially more rapidly than others, and these may be predictable from observations before the trial begins.

Objectives: To generate an algorithm that finds a combination of sub-measures that would change most rapidly for each subject, thus potentially increasing the power or reducing the sample size required for a treatment comparison.

Methods: Data were available on 488 patients from four clinical trials conducted by New England ALS consortium. Depending on the trial, measures were available either monthly or bi-monthly. Three methods to develop an individualized score were attempted. We considered measurements used to screen patients and those made during the first two months of each clinical trial as if they were from a run-in period, and subsequent measurements as if they were from the treatment period. The first method used the sum of the scores for muscles with the largest drop in value during the run-in period as the individualized measure. The second used Lagrange multipliers to find the weighted sum of the sub-measures that dropped the fastest during the treatment period subject to the constraint that the standard deviation of this weighted sum was constant, and that the weights were linear combinations of the measurements made during the run-in period. The third used weights based on baseline values rather than run-in values. We evaluated the methods using Lagrange multipliers by splitting the data into two subsets, a training sample used for determining the coefficients and a validation sample used for evaluating the method.

Results: The first method did not produce an advantage. The second method gave gains when applied to the training sample but not the validation sample. The third method using Lagrange multipliers applied to the baseline values resulted in a measure that reduced the sample size required to determine an effect of predetermined magnitude by 23% when applied to the validation sample.

Conclusions: Modest but important reductions in sample size are possible using this strategy. Using summary measures offers the prospect of combining qualitatively different measures (such as strength and score on a rating question) into a single measure that declines rapidly for an individual patient. The discrepancy found between the application of a measure to the training and validation set implies that a very large database is needed to train this algorithm. The estimated coefficients may provide useful information as to the way the disease symptoms progress.

P102 BULBAR FEATURE ASSESSMENT: A MULTILEVEL APPROACH

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Background: People with bulbar features of ALS are likely to experience a mixed spastic-flaccid dysarthria, a speech condition characterized by reduced intelligibility, at some point during disease progression. Existing staging systems are descriptive of current levels of speech functioning in people with ALS (PALS); however, they are not sensitive to early symptom detection. The bulbar neurons are prominent in differential diagnosis; therefore early identification of bulbar symptoms is crucial to facilitating diagnosis and increasing the earliest symptomatic PALS participation in pharmaceutical trials. In addition, early speech symptom indicators may assist in the development of effective treatments designed to sustain functional intelligibility.

Objectives:

1. Establish an assessment of bulbar features through a longitudinal study of clinical evaluations involving more than 300 PALS.
2. Identify procedures sensitive to identification of very early bulbar symptoms.
3. Demonstrate use of bulbar assessment for clinical trials.

Methods: In this session, a comprehensive assessment of bulbar attributes, comprised of selected levels of evaluation specificity, is tested: 1) Level 1, easily completed by clinical staff, is an assessment of speaking rate in sentences; B) Level 2, involves increased specificity in evaluation, through perceptual/acoustic measures of speech by a speech-language pathologist; 3) Level 3, involves motion analysis of the tongue during speech using 3D articulography; 4) Finally, the three levels of assessment are implemented as part of bulbar function monitoring during a clinical trial.

Results: Data indicate that speaking rate is predictive of loss of speech intelligibility in PALS. Specific bulbar features include upper motor neuron and/or lower motor neuron characteristics observed in oral-facial movements during functional activities (speech and swallowing). Data supporting the use of speaking rate as a measure of neurodeterioration in PALS are presented. A method was developed to quantify the diversity of tongue movement patterns for PALS spoken utterances. These findings are now being compared with reference data to gauge the degree of impairment of tongue function in speakers with neurologically-based speech impairments, e.g. ALS. Results from computer based acoustic analysis indicate PALS demonstrates increased pause time and speech time during sentence production. Strategies to implement the bulbar assessment in a clinical trial, including frequency and appropriate timing of data collection, are presented.

Discussion: This project involves an effort to standardize assessment of bulbar function. Assessment and intervention strategies are included. Audio and video samples will be used for illustration of acoustic, perceptual and movement characteristics. Preliminary findings of this novel multilevel bulbar function assessment will be compared with the most common existing measures as applied during a clinical trial.

P103 STUDY OF RILUZOLE IN A PROSPECTIVE COHORT OF 501 SUBJECTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Riluzole prolongs survival in amyotrophic lateral sclerosis (ALS) as demonstrated in two clinical trials. An effect on functional measures was not shown in these two trials.

Objective: To examine the effect of riluzole on functional outcome measures in a database population of 501 ALS subjects enrolled in three recently completed clinical trials performed by the Northeast ALS Consortium (NEALS).

Methods: The randomized, double-blind, placebo-controlled clinical trials of topiramate, creatine and celebrex in ALS subjects were conducted from 1998 to 2004. Five hundred and one subjects diagnosed with probable or definite ALS were included in our analysis. We excluded the subjects randomized to topiramate because treatment may have accelerated disease course. Treatment duration was 12 months for participants in the topiramate and celebrex trials and six months for the creatine trial. Disease progression was measured with the Maximum Voluntary Isometric Contraction (MVIC) arm, grip megascores, the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) and vital capacity in all three trials; MVIC leg strength only in the celebrex trial. All subjects who took riluzole at baseline were considered as 'riluzole users'.

A Kaplan-Meier survival analysis based on riluzole treatment at the enrolment visit was performed. To control for baseline covariates, a Cox proportional hazards model was used. The rate of decline of different outcome measures was measured in slopes and compared in the riluzole and non-riluzole groups. The difference was tested with *t*-tests, and each subject's slope was estimated from the mixed model.

Results: Of the 501 ALS subjects, 318 took riluzole (riluzole group) whereas 183 did not receive riluzole at any stage of their enrolment period (non-riluzole group). Survival increased approximately 19% in the riluzole group compared to the non-riluzole group, but the difference was not significant ($p=0.32$). The hazard function in the riluzole group did not change after controlling for various baseline covariates. There was no significant difference between the functional outcome measure slopes of the non-riluzole and the riluzole groups. After controlling for the site of symptom onset, the MVIC arm megascore of subjects in the riluzole group (slope estimate$=-0.091$) declined more rapidly than the non-riluzole group (slope estimate e$=-0.079$, $p=0.02$).

Conclusions: In a cohort of 501 ALS subjects, riluzole was not found to influence rate of decline in muscle strength, pulmonary function or ALSFRS. A limitation of our study is that the three clinical trials were not randomized to riluzole use.

P104 HEPATITIS: AN ADVERSE EVENT DURING THE TREATMENT OF ALS WITH RILUZOLE OF HETEROGENEOUS ORIGIN

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Background: Riluzole has been licensed for ALS treatment since 1997. It is a well-tolerated drug with a potential and rare liver toxicity, inducing cytolytic hepatitis which rapidly disappears after treatment withdrawal.

Objectives: To describe the spectrum of hepatitis during the treatment by riluzole in ALS.

Methods: In the last two years, nine patients (out of 300) were identified with a significant increase of either ALAT, ASAT, gamma glutamyl transferase (GGT) or alkaline phosphatase that led to them stopping riluzole or to decreasing their daily dosage.

Results: Seven males and 2 females aged from 42 to 78 years (mean 62 years) developed hepatitis. In one patient (case 1), sustained GGT was also associated with alcohol abuse. In three cases (cases 2, 3 and 4), ALAT and ASAT were increased above the 5N limit, corresponding well to previously described cases with cytolytic hepatitis. In five cases (cases 5 to 9), there was a significant increase of GGT (>5N except in 1 case >3N), without increase in ALAT or ASAT and the final diagnosis was cholestatic hepatitis. In cases 1, 2, 5 and 6 withdrawal of riluzole was not followed by complete normalization of the biological parameters as GGT after several months remained at 2N. In all the other cases biology is now normal.

Discussion: Our group of patients show that not only ALAT and ASAT should be screened during the course of ALS treatment by riluzole. While adverse biological events are not severe and do not appear to have any clinical consequence, the persistence of a GGT increase in four cases underlines the possibility that some ALS patients may have a liver disorder in parallel. Such liver abnormalities have been described in the literature but their origins remain undetermined. Recently the HFE gene has been described as a genetic susceptibility factor for ALS. It would be of interest to look for these mutations in our patients especially when the liver biology becomes abnormal.

Conclusion: Riluzole treatment remains well tolerated in ALS. Our cases underline the importance of a systematic check of GGT and alkaline phosphatase in parallel with ALAT and ASAT, which is not recommended to date by regulatory authorities. A liver disorder may evolve in parallel with ALS in some patients. However, we cannot suggest that such a disorder could play a role in ALS pathogenesis.

P105 THE RATE OF WEIGHT LOSS AND NOT BODY MASS INDEX IS A STRONG PROGNOSTIC FACTOR IN ALS

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Background: ALS prognosis is related to several factors, among which malnutrition seems to be one of the most relevant. However, there is no consensus about the best measure of malnutrition in ALS, which can be used in order to establish the necessity to start enteral nutrition via PEG/RIG.

Objective: To assess the indicators of malnutrition and outcome in a series of ALS patients followed up in our centre.

Methods: All patients regularly followed up in our centre, seen for the first time in the period 1 January 1996–30 June 2000, were included in the study. The patients were seen every three months and weight was evaluated at each visit. The patients also underwent ALS-FRS. At the first visit, health body weight (HBW) was recorded. The following parameters (taken at diagnosis, at the six-month and at the 12-month visits) were considered for analysis: weight, BMI, weight loss or gain with respect to HBW, weight loss or gain with respect to the preceding visit. Possible confounding factors were considered, i.e. age, gender, type of onset (bulbar vs. spinal), presence of bulbar signs at the time of the visit, FVC% at the time of the first visit, PEG/RIG, non-invasive ventilation, and tracheostomy. Survival analysis was performed by the Kaplan-Meier method, and differences were assessed with the log-rank test. Cox's multivariate analysis, stepwise, was performed to determine the independent prognostic factors.

Results: A total of 189 patients were included in the study (99 males and 90 females; mean age 61.5 years (SD 11.1), 63 bulbar onsets and 126 spinal onsets). The mean BMI when healthy was 26.5 (SD 3.9). The mean BMIs at diagnosis, at the six-month, and at the 12-month visits were 25.4 (SD 3.5), 24.6 (SD 3.8), and 24.0 (SD 3.7), respectively. At the six-month and 12-month visits, 13 and 19 patients, respectively, had a BMI lower than 18.5. The mean weight loss at the six-month and 12-month visit was 7.4% and 9.5%, respectively. The median survival from onset was 963 days. In univariate analysis, factors significantly related to outcome were age, site of onset, and FVC%, weight loss over 6% at the 12-months visit ($p<0.001$) and rate of weight loss (i.e. mean weight loss per month) from onset to the six-month and 12-month visits. In multivariate analysis, the independent factors were mean weight loss per month ($p<0.0001$), FVC% ($p=0.0013$), enteral nutrition ($p=0.003$), and age ($p=0.017$).

Conclusions: According to our data, the best indicator of malnutrition in ALS is the rate of loss of weight during the course of the disease. BMI did not result in a significant parameter for establishing the necessity of performing PEG, since several patients had a BMI over 18.5 when they had already lost more than 10% of their weight. Therefore, weight loss should be included in future trials as a reliable outcome parameter.

P106 SEVERE PROGRESSION OF AMYOTROPHIC LATERAL SCLEROSIS AFTER INTERVERTEBRAL DISCECTOMY

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Background: The diagnosis of ALS can be mimicked in the initial phase by some other disorders such as intervertebral disc prolapse in the cervical spine with compressive radiculopathy/myelopathy.

Objectives: The aim of this study was to determine the frequency of ALS patients with intervertebral disc prolapse in the cervical spine with compressive radiculopathy and/or myelopathy and to determine specifities in the course of ALS in these patients.

Methods: The study population comprised 600 ALS patients diagnosed in the period 1992–2002 at The Institute of Neurology, Belgrade. All the patients fulfilled the El Escorial criteria of probable or definite ALS.

Results: Prolapse of intervertebral disc in the cervical spine with compressive radiculopathy and/or myelopathy was diagnosed in 47 (7.8%) ALS patients. Among them were 27 patients with severe pain in cervical spine and persistent paraesthesia in dermatomes C4/C5, C5/C6 or C6/C7, as initial symptoms. Intervertebral disc prolapse in the cervical spine with compressive radiculopathy and/or myelopathy was diagnosed within a period of 6–12 months, prior to the diagnosis of ALS. Wasting of the arm muscles was variable at the time of onset. After discus hernia with compressive radiculopathy and/or myelopathy had been diagnosed, seven patients underwent surgical treatment while 20 were conservatively treated. The mean age in the first subgroup was 49.1 ± 10.4 years, and in the second subgroup 51.3 ± 9.8 years ($p>0.05$). Patients from both subgroups developed progressive wasting in the arms and later of the limbs as well as bulbar muscles, and diagnosis of ALS was confirmed. Life span after the first symptoms had appeared in ALS patients who underwent discectomy was 21.1 ± 1.6months. In ALS patients who had not been surgically treated it was 28.5 ± 3.0 months. Comparison of these two subgroups of ALS patients showed significantly shorter lifespan in patients who underwent discectomy ($p<0.01$).

Conclusion: The results of our study showed that discectomy in the cervical spine seems to precipitate rapid progression of ALS.

P107 MILD EVOLUTION IN A SUBSET OF ALS/MND CASES WITH BULBAR ONSET: A DIFFERENTIAL DIAGNOSIS?

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Background: ALS is a severe disorder with, in approximately 25 to 30% of cases, a bulbar onset. In the latter presentation, the prognosis is the worst. In recent years, some cases of ALS/MND with a favourable evolution initially masquerading as ALS have been described.

Objectives: To describe a series of ALS/MND cases with bulbar onset and mild evolution after one to six years.

Methods: In the last six years, eight patients were identified with a bulbar onset form of MND for whom a diagnosis of possible, probable or definite ALS was made. They were followed according to the requirements for ALS patients in our MND clinic.

Results: Three males and 5 females aged from 32 to 72 years (mean 55 years) were studied. Diagnosis of definite ALS was made in two cases, probable in two cases and possible in four cases. Symptoms at onset were dysphonia (three cases), dysphagia (three cases) and a mix of bulbar symptoms (two cases). In three cases EMG showed a denervation restricted to the bulbar level. In one case the disease was a pure lower MND. In six cases, upper motor neuron signs were present at three levels (bulbar, upper and lower limbs). Three patients clearly improved one year after diagnosis. One patient is stable after five years. For the four other cases, ALSFRS is declining at a maximum rate of 1 point per year. None of those cases are familial. Differential diagnosis used comprehensive biology, brain MRI, and lumbar puncture. For two males, a detection of the Kennedy's CAG expansion was performed and was negative. The third male had clear spasticity and this test was not proposed.

Discussion: While ALS is known to have a severe progression, slow ALS cases may represent 10 to 20% of the cases. The evolution of paralysis is a part of the diagnosis criteria, making it likely that a diagnosis of ALS may be refuted for a patient with mild evolution. We cannot propose, to date, another diagnosis than bulbar MND of slow or atypical ALS for these patients.

If one considers that the diagnosis 'ALS' cannot apply to those patients, this would imply that the diagnosis of ALS can only be an 'a posteriori' one. Even if this is the way of proceeding for the majority of neurologists, and if this is possibly the right way to proceed, an 'a posteriori' definition for a given concept is not scientifically correct.

Conclusion: Our eight bulbar onset ALS/MND cases either improved, did not worsen, or slowly evolved. It is tempting to propose an expansion of the concept of MND. Undoubtedly, this leads to being perhaps more careful than before when announcing a diagnosis or a prognosis to our patients.

P108 THE CLINICAL SPECTRUM OF SPORADIC ADULT-ONSET UPPER MOTOR NEURON DISEASE

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Background: A syndrome of isolated upper motor neuron (UMN) degeneration was first described by Charcot in 1865 and was named primary lateral sclerosis (PLS) by Erb in 1875. It has been much debated whether PLS exists as a separate disease entity. Diagnostic criteria for PLS were proposed by Pringle et al. in 1992 and require progressive symmetrical spinobulbar spasticity of at least three years duration with adult onset, no clinical and at most minor electrophysiological evidence of lower motor neuron (LMN) involvement, a negative family history and exclusion of other causes.

Objectives: To explore the full clinical spectrum of sporadic adult-onset UMN disease.

Methods: We performed a nationwide search for patients with adult-onset sporadic UMN disease in the Netherlands. Inclusion criteria were: a gradually progressive UMN syndrome, adult onset, duration of three years or more, exclusion of ALS with needle EMG after at least three years duration, and absence of a family history suggestive of hereditary spastic paraparesis (HSP). Exclusion criteria were: clinical evidence of generalized LMN involvement, presence of cerebella, extrapyramidal, or sensory signs and evidence for other causes.

Results: Ninety-seven patients were included (median age 58 years, range 22–80 years): 82 had onset in the lower limbs, four in the upper limbs, eight in the bulbar region and three simultaneously in different regions. At inclusion, 36 patients had bulbar involvement, 48 had involvement of upper limbs and 93 of lower limbs. Patients differed in the number of affected body regions, the chronological order of involvement of the different regions and the degree of symmetry. In some patients UMN signs were strictly unilateral or restricted to one region (lower limbs or bulbar). A transient phase of isolated lower limb involvement could be as long as 18 years. Minor EMG abnormalities were found in 40 of 78 re-examined patients.

Conclusions: Patients with sporadic adult-onset UMN disease show a wide spectrum of clinical presentations, with variable rates and patterns of progression. The Pringle criteria for PLS may need revision to include variants of UMN disease other than symmetrical spino-bulbar spasticity. Clinical distinction with sporadic HSP remains problematic as long as DNA tests for most forms of HSP are not available. The presence of mild LMN signs in some patients is of uncertain significance, but could support the view that PLS is a clinical variant of ALS.

P109 PRIMARY LATERAL SCLEROSIS AND AMYOTROPHIC LATERAL SCLEROSIS DISPLAY DIFFERENCES AT DISEASE ONSET AND DURING FOLLOW-UP THAT CAN HELP DIFFERENTIATE THE TWO ILLNESSES

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Background: Primary lateral sclerosis (PLS) is a neurodegenerative disorder involving the upper motor system. Its existence as a separate entity from ALS is still controversial.

Objectives: To compare patients with PLS or ALS for differences in symptoms or signs at disease onset and during follow-up to determine which features best differentiate these two entities.

Methods: Patients with PLS or ALS followed in the MND clinic were included for analysis. Mann-Whitney tests were used to look for significant differences between the two groups.

Results: Seven hundred and thirty-six patients with ALS and 47 patients with PLS were used for this analysis. The male to female ratio was not significantly different between the two groups. ALS patients were significantly older than PLS patients at symptom onset (59.17 vs. 54.43 years, $p<0.005$). Bulbar onset was more common in ALS patients in that 24% had dysarthria and 8.2% had dysphagia at symptom onset compared to 14.9% of PLS patients who had dysarthria and 0% presented with dysphagia ($p<0.05$). At presentation, paresthesias were not significantly different between the two groups ($p=0.37$). Fasciculations and cramps were only a presenting feature in ALS patients with 8.8%, $p<0.05$ and 6.3%, $p<0.05$, respectively. Weakness was significantly more common as a presenting symptom in ALS patients at 56.7 vs. 42.6% ($p=0.05$). There was a trend towards wasting being more common as a presenting sign in ALS patients at 6.1% ($p=0.053$). Stiffness was significantly more common as a presenting symptom in PLS patients vs. ALS (46.8% vs. 3.3%, respectively, $p<0.001$). With respect to follow-up, bulbar symptoms were significantly more common in ALS patients than PLS, at 87.5% vs. 74.5% ($p<0.005$). Limb wasting was rare in PLS patients, at 4.3% vs. 99.2% in ALS patients, $p<0.001$. The presence of pyramidal and sensory symptoms, however, was not significantly different between the two groups. Clinically overt dementia was significantly more prevalent in ALS patients vs. PLS, at 6.5% vs. 0%, $p<0.05$. Trauma was more commonly encountered in PLS patients, at 17% vs. 7.3 %, $p<0.05$. There was a trend for rheumatological disease to be more common in ALS patients, at 5% vs. 0% ($p=0.096$). Autoimmune disorders were more common in PLS patients, at 4.3% vs. 0.4% in ALS patients ($p<0.002$).

Conclusions: PLS patients differ from ALS patients in their symptom onset site as well as their ongoing symptoms. Stiffness is more often a presenting symptom of PLS while fasciculations, cramps and wasting are uncommon in PLS. Over time, PLS patients rarely develop limb wasting compared with ALS patients.

P110 ASSESSMENT OF RESPIRATORY FUNCTION, SLEEP QUALITY AND ACTIVITIES OF DAILY LIVING IN PLS PATIENTS

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Background: Primary lateral sclerosis (PLS) is a rare neurodegenerative disorder involving upper motor neuron (UMN) degeneration with slow symptom progression of lower extremities or bulbar region. A paucity of respiratory function and sleep data is reported for PLS patients. With characterization of PLS respiratory involvement, discrete differences in respiratory function between UMN and lower motor neuron involvement may be identified.

Objective: To assess the impact of PLS on sleep and pulmonary function using spirometry, St. George Respiratory Questionnaire (SGRQ), Pittsburgh Sleep Quality Index (PSQI), and ALS Functional Rating Scale-Revised (ALSFRS-R), compared with ALS control subjects.

Methods: Recruitment of 11 PLS and 11 ALS patients was through PLS Patient Conference and MND clinics at one site in the USA. Respiratory function was measured as forced vital capacity (FVC). ALS controls matched FVCs of PLS subjects. Subjects self-administered three questionnaires: SGRQ measures respiratory involvement, PSQI measures sleep quality and ALSFRS-R. Validated electronic databases scored SGRQ PSQI and ALSFRS-R. Student's two-tailed *t*-tests analyzed data of PLS vs. ALS subjects.

Results: PLS subject FVCs are not significantly different from those of ALS controls (mean 93.45 and 79.45, respectively, $t=1.81$, $p=0.086$). Duration of disease is not significantly different between PLS and ALS (mean 87.4

and 54.1 respectively, $t=1.53$, $p=0.07$) in our study population. ALS subjects had significantly higher SGRQ symptoms score ($t=2.47$, $p=0.022$). ALS subjects had a significantly higher total SGRQ score ($t=2.12$, $p=0.046$). SGRQ activity and impact components (demonstrating quality of life) are not significantly different in PLS compared with controls ($t=1.67$, $p=0.11$; $t=1.57$, $p=0.13$, respectively).

Both groups had PSQI total values above 5, signifying 'poor sleep'. None of the PSQI measures of sleep quality are significantly different for PLS compared to ALS; however, the PLS group had shorter total sleep time than ALS (mean 0.73, 1.27 respectively, $t=1.27$, $p=0.22$), reduced sleep efficiency (mean 0.56, 0.91 respectively, $t=0.86$, $p=0.40$), and used more sleep medications (mean 0.64, 0.27 respectively, $t=0.88$, $p=0.39$). None of the ALSFRS-R measures were significantly different in PLS vs. ALS.

Discussion: ALS and PLS subjects are well matched by FVC to eliminate disease severity as a cause of differences between the two disease groups. PLS subjects were symptomatic longer than equivalent ALS, suggesting respiratory impairment is seen much earlier and is more severe compared to PLS. PLS subjects had fewer respiratory complaints then ALS, suggesting there may be unmeasured factors playing a role in neuromuscular control of respiration not estimated by FVC measurement alone. Based on publications, FVC can underestimate the degree of respiratory impairment in ALS. Of note, PLS subjects reported a reduction in overall sleep quality compared to ALS, even though they used more sleep promoting medications.

P111 FLAIL-LIMB VARIANT OF MOTOR NEURON DISEASE HAS EVIDENCE OF CENTRAL AND PERIPHERAL NERVE DYSFUNCTION

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Background: Motor neuron disease (MND) is a progressive neurodegenerative disorder of corticomotoneurons and anterior horn cells (AHC), with median survival of approximately two years. In 20% of MND patients, survival is prolonged greater than five years. A specific group of MND patients with increased survival is the flail limb variant of MND, also known as 'man-in-the barrel' syndrome or brachial amyotrophic diplegia syndrome (BAD). This variant of MND is characterized by proximal onset of muscle weakness and wasting in the upper limbs with initial preservation of bulbar and lower limb function. The aim of the present study was to further define the clinical phenotype of the flail limb variant of MND, and to explore disease pathophysiology through the application of novel nerve excitability and transcranial magnetic threshold tracking techniques.

Methods: Clinical data, structural imaging and conventional neurophysiological studies were collected for patients with the flail limb variant of MND. Inclusion criteria included a pattern of weakness confined to the proximal aspects of the upper limbs for at least two years. The results of neurophysiological testing were used to exclude patients with multifocal motor neuropathy with conduction block and patients with other forms of demyelinating neuropathy. Patients were followed with the amyotrophic lateral sclerosis functional rating scale-revised (ALS-FRS-R). Nerve excitability testing was undertaken in a proportion of patients.

Results: In total, nine patients with the flail limb variant of MND were detected from our database. Their mean age at disease onset (60.3 years) was similar to patients with other variants of MND. The male: female ratio was significantly increased in the flail limb variant of MND (8:1 vs. 1.6:1, $p<0.05$). Mean duration of illness was also significantly longer in the flail limb variant of MND (76.3 vs. 20.8 months, $p<0.05$). Lower limb symptoms developed in two (29%) patients 4.9 years after symptom onset, while bulbar symptoms developed in three (43%) patients, 36 months after symptom onset. Respiratory symptoms occurred in two patients, 31 months after symptom onset and both patients required non-invasive ventilatory support. Transcranial magnetic stimulation revealed absence of early intracortical inhibition consistent with cortical hyperexcitability in the absence of clinical upper motor neuron signs. Nerve excitability testing demonstrated evidence of activity dependent conduction failure, suggesting the presence of axonal membrane Na^+/ K^+ ATPase pump dysfunction.

Conclusion: The flail limb variant of MND is a distinct clinical type of MND with male predominance and longer survival. Cortical excitability is increased, similar to patients with other forms of MND, arguing against isolated lower motor neuron involvement.

P112 CLINICAL AND NEUROPATHOLOGICAL STUDY OF A SERIES OF ALS-DEMENTIA SYNDROME PATIENTS: ANALYSIS OF OUR EXPERIENCE

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Background: ALS has traditionally been considered a neurodegenerative disease that selectively affects the motor neurons. However, some patients show evidence of cognitive impairment, which in most cases is mild and appears in the advanced stages of the disease. Dementia is nevertheless severe in a subset of patients and appears at the same time as motor deficits, and is therefore an

ALS-dementia syndrome (ADS). We present the findings for 13 patients with ADS.

Objectives: Clinical and neuropathological characterization of the ALS-dementia syndrome.

Patients and methods: We identified 13 individuals with clinical ADS criteria in the database of our ALS Unit (417 patients). They were neuropsychologically assessed. Screening for SOD1 gene mutations was carried out. A necropsy was performed on six patients.

Results: The prevalence of ADS was 3.1%. The average age of onset was 63.3 years and the average survival time was 27.9 months. Bulbar forms predominated. There was a fronto-temporal dementia (FTD) profile in 11 patients. The atypical clinical findings observed were supranuclear gaze paralysis in one patient and voluntary lid closing inability (eyelid 'apraxia') in another. No mutations in SOD1 gene were found.

As regards neuropathological findings, we observed varying degrees of spongiosis in layers I/II of the frontal cortex, neuronal loss and presence of subcortical gliosis in all patients. We identified ubiquitin-immunoreactive inclusions in the cortical neurons in five patients. In the spinal cord, neuronal loss and gliosis in the ventral horn was most severe in the cervical region in three patients, in the dorsal region in two, and the lumbar region in one. We found atrophy of the ventral roots and severe motor neuron loss in the hypoglossus nucleus in all patients. Three patients presented substantia nigra neuronal loss. Neuritic plaques in the hippocampus were identified in two patients.

Discussion: These findings suggest that ALS could be a multisystemic disorder with a limited phenotypical expression. The most striking clinical finding was the presence of ocular-motor abnormalities in two patients of the series. The neuropathological abnormalities present in ADS would represent a 'continuum' with other neurodegenerative diseases.

Acknowledgement: FIS 02/0648

P113 MUSCLE AND JOINT PAIN IN MND/ POST-POLIOMYELITIS (PPS)

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Introduction: Post-polio syndrome (PPS) is the term most commonly used to describe the new difficulties which may occur many years after recovery from paralytic poliomyelitis. Although many symptoms may be reported by these patients, the most commonly reported are new weakness, fatigue, and pain. These three symptoms have been used to define PPS.

The cause of PPS remains unclear, although the most widely accepted hypothesis, proposed by Wiechers and Hubbell, attributes these symptoms to a distal degeneration of axons in the greatly enlarged motor units that developed during recovery following acute paralytic poliomyelitis.

Currently, it is unclear how the symptom of pain, which is uncommon in the classical motor unit disease such as amyotrophic lateral sclerosis, is related to disease of the motor unit. Although in ambulatorial assessment pain is frequently reported, pain can be due a variety of causes. It can be caused by muscular abnormalities, joint and soft tissues abnormalities, and other superimposed neurological abnormalities. Possible causes for muscular pain include muscular overuse, muscular cramps, fasciculation, and fibromyalgia.

Objectives: The purpose of this study is to demonstrate the pain features in PPS ambulatorial patients.

Patients and methods: Between March 2003 and June 2004, 129 patients (81 female, 48 male, age 18 to 72 years, mean 39.9 ± 10.07 years), with a confirmed diagnosis of PPS according to consensus criteria, attended the outpatient ambulatory neuromuscular disease of the School of Medicine of University of São Paulo. All patients submitted to neurological evaluation, blood and urine tests and their pain was evaluated using the Pain Analogical Scale (PAS).

Results: Joint pain was found in 79.8% (103) and muscle pain in 76.0% (98) of patients. The PAS scores ranged from 0 to <10. A pain intensity score of 4 was found in 10.9%, and a score 5 in 14.7% (moderate pain). 14.7% had a pain score of 7 and 18.6% a score of 8, representing moderate to high pain. The distribution of pain found was: 8.5% (12) no pain; fibromyalgia 2.3% (3); headache 2.3% (3); cervical pain 2.3% (3); shoulder pain 7.0% (9); upper limb pain 19.4% (25).

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 127–137

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THEME 6 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P114 BRAIN METABOLITES IN ADVANCED ALS: A LONGITUDINAL ^{1}H-MRS STUDY

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Background: Standard MRI in patients suffering from amyotrophic lateral sclerosis (ALS) provides only little information on stage of disease and its progression. Besides the description of hyperintense signal alterations of the corticospinal tract (CST), which have to be considered as non-specific, there are variable findings of disproportionate enlargements of the central sulcus.

Objectives: Several approaches have been made to investigate whether or not proton magnetic resonance spectroscopy (^{1}H MRS) may serve in detecting upper motor neuron degeneration. Nevertheless, decrease of concentrations of N-acetyl-aspartate (NAA) and increase of concentrations of creatine (Cr) and choline (Cho) have been observed in patients with ALS compared to healthy controls, while longitudinal decrease of NAA-, Cr- and Cho-concentrations were detected during the progression of disease. The objective of this study was to investigate the longitudinal development of the concentration of brain metabolites in advanced ALS.

Methods: Ten patients with definite ALS underwent standard single-voxel ^{1}H MRS of the left- and right-hemispheric motor cortex as well as the white matter including the CST in a six month follow-up series of three measurements. MRS data were co-registered with tissue-segmented MRI data to obtain concentrations of the metabolites while fully automated post-processing included spectral fitting of the peak areas of NAA, Cr, and Cho.

Results: In a cross-sectional analysis, the NAA/(Cr + Cho) ratio of the motor cortex was reduced as expected, a reflection of decreased NAA and increased Cr and Cho. All patients showed a marked decline of NAA in the motor cortex areas with nearly stable concentrations of Cr and Cho in the longitudinal follow-up. In contrast, neither the concentration of NAA nor the NAA/(Cr+Cho) ratio in white matter areas showed comparable dynamics.

Conclusions: As reported in the literature, the NAA/(Cr + Cho) ratio of the motor cortex was shown to be in a sustained decline corresponding to a decrease of NAA as a neuron-specific marker. As a result, NAA seems to be the most valuable candidate as a surrogate marker for *in vivo* detection of disease progression and staging.

P115 MR-PATHOLOGICAL COMPARISON OF CERVICAL AND THORACIC SPINAL CORD IN DIFFERENT MOTOR NEURON DISEASES

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Background: Defined types of motor neuron diseases (MND) differ in clinical, electrophysiological, genetic, and histopathological findings, but phenotype differentiation often fails to elude the distinct neuropathological pattern of vulnerability. Furthermore, besides marked changes in lower motor neurons, electrophysiological and neuropathological investigations can show corticospinal tract abnormalities and dorsal column alterations of the spinal cord in MND.

Objectives: This MRI study was performed to evaluate *in vivo* alterations of the spinal cord in defined subgroups of MND, i.e. sporadic amyotrophic lateral sclerosis (ALS), sporadic adult-onset lower motor neuron disease (LMND), and spinobulbar muscular atrophy (Kennedy's disease, KD).

Methods: Standard T1- and T2-weighted MRI examinations of cervical and thoracic spinal cord in 39 ALS, 19 LMND and 19 KD patients were studied with respect to the presence of spinal cord signal changes. The thickness of the spinal cord both at cervical (at C2) and at thoracic level (at T4) was measured, and differences were analysed between MND groups compared with a control collective (n=96).

Results: We found no signal alterations at cervical and thoracic spinal cord in all groups investigated. The diameters of cervical and thoracic spinal cord in ALS and LMND patients showed no differences to controls and to each other. In KD patients, significant atrophy of the upper spinal cord compared to the other groups was detected both at cervical and thoracic level.

Conclusions: In summary, no significant changes in thickness in spinal cord diameter at both levels were observed in ALS and LMND, whereas marked atrophy of the upper spinal cord was seen in KD. This finding seems

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045041

to be a characteristic feature in KD, probably due to the KD-associated central and peripheral distal axonopathy.

P116 MRI-VOLUMETRY OF THE AMYGDALA IN ALS

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Background: The evidence for extra-motor involvement in non-demented patients with ALS has been provided in neuropathological, neurophysiological, electrophysiological and neuroimaging studies. Neuropathological studies have elicited a neuronal loss in the amygdala.

Objectives: The aim of this study was to assess a possible decrease of the amygdala volume by *in vivo* MRI-volumetric measurements.

Methods: Twenty-two patients with a clinical diagnosis of ALS according to the revised El Escorial criteria and 22 age-matched healthy controls were included in the study. None of them showed any relevant cognitive or behavioural deficit. The amygdala volumes and whole brain volumes were measured by 'region-of-interest-based volumetry' (MReg-Software, L. Lemieux, http://www.erg.ion.ucl.ac.uk/). The mean amygdala volume and the ratio of amygdala to whole brain volume were compared between patients and controls.

Results: A decreased volume of the amygdala could be found in the patient group compared to the controls for the mean amygdala volume as well as for the ratio of amygdala to whole brain volume. However, these results did not reveal a statistically significant difference.

Conclusions: By MRI-volumetry, a statistically non-significant trend to decreased amygdala volumes in ALS patients was shown. An investigation into an association between MRI-volumetric alterations of the amygdala and psychopathological changes in ALS is the matter of a current study, in ALS patients with behavioural and cognitive deficits.

P117 STRUCTURAL CHANGES IN THE CORTICAL MOTOR SYSTEM OF ALS PATIENTS? DATA OF AN FMRI STUDY

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Background: Cortical reorganization following lesions in the motor cortex is a well-known mechanism of neuronal plasticity. The aim of the study was to investigate changes of cerebral functions during the course of the progressive degeneration of the motor system of ALS patients.

Objectives: With the help of functional magnetic resonance imaging (fMRI) we examined cortical activity during actual and imagined hand movements twice in six months in ALS patients and compared the data to those of healthy controls.

Methods: Thirteen patients with sporadic ALS and 14 healthy controls were asked to perform tasks of a grip movement in each hand and both hands together (predefined power of 5N) and to imagine the same action without any actual movement of the hand. The tasks were set in a randomized block design alternating with rest periods. The actual force applied by the participant was recorded simultaneously. Beforehand, the participants had been trained in a scanner dummy to perform the tasks properly. The actual experiment took place in a 1.5 Tesla MRI scanner. Differences in the BOLD signal were analysed with Statistical Parametric Mapping (SPM2, Wellcome Institute of Cognitive Neurology, London, UK).

Results: For ALS patients as well as for healthy controls we found BOLD-signal changes in sensomotory and supplementary (SMA) areas. In contrast, for the imagined hand movements there were signal changes in premotor areas without any signal changes in areas responsible for executive functions. In ALS patients compared to healthy controls there was evidence of differences in functional BOLD-signal changes in cortical and subcortical areas for the imagined movement. Furthermore, changes during the course of the disease over six months were found.

Conclusions: For actual hand movements we found comparable cortical activity for ALS patients and for healthy controls. For the imagined hand movements patients show a different pattern of cortical activation from controls. This might be an indicator of a progressive process of cortical reorganization in the course of the degeneration of the motor system of patients with ALS. The longitudinal data give further insight into the processes of reorganization.

P118 DIFFUSION TENSOR IMAGING (DTI) AND MAGNETIC RESONANCE SPECTROSCOPY (MRS) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): PREDICTOR INDEX OF INVOLVEMENT OF UPPER MOTOR NEURON (UMN)

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Background: The evidence of UMN dysfunction in ALS is not easily evaluated with either objective markers for discrete clinical UMN signs or for severe simultaneous lesions of the lower LMN. Recently neuroimaging is more often applied with MRS and DTI.

Objectives: The aim of this study was to contribute to the evaluation of the role of MRS and DTI.

Methods: 27 patients (18 male; 58 ± 10 years of age) and 15 healthy controls (HC) (three male; 45 ± 14 years of age) underwent MRI examination. The patients were diagnosed according to El Escorial criteria and re-allocated for statistical purposes into definite/probable (D-ALS) ($n=15$; 8 male) and possible/suspected (S-ALS) ($n=12$; 10 male). MR studies were performed on 1.5 T equipment. DTI indices were calculated (averaged apparent diffusion coefficient, avADC; fractional anisotropy, FA) sampling from grey matter of motor cortex (GM) to brainstem; single voxels MRS were localized in bilateral central regions. Parametric and a non-parametric test were performed for statistical analysis (*t*-test, Mann-Whitney test, ANOVA; Bonferroni's correction; Cuzick's χ^2 test; Pearson and Spearman coefficients).

Results: In patients with D-ALS the mean values of FA were lower and avADC higher than HC, both statistically significant, in bilateral GM and right white matter (WM). Also ANOVA testing considering the three groups (D-ALS, S-ALS and HC), showed statistically significant values for FA and avADC in GM and WM. Bonferroni's correction revealed: for FA, statistically significant differences in D-ALS vs. HC in bilateral GM and right WM; S-ALS vs. HC in left GM. For avADC there were statistically significant differences in D-ALS vs. HC in bilateral GM and right WM; S-ALS vs. HC on the right in GM and WM. In D-ALS vs. S-ALS there was no statistically significant difference. In D-ALS and S-ALS patients the MRS data, compared to normal values, on both sides showed statistically significantly lower values of the ratio of N-acetylaspartate (NAA) /creatine/ phosphocreatine (Cr) and higher values of the ratio of myo-inositol (mI)/(Cr). Also the choline values were higher in ALS than control, but at the limits of statistical significance.

Conclusions: The main difference between the previous studies and the current investigation was the increased avADC and decreased FA in D-ALS compared to HC in GM of motor cortex, associated in MRS testing to reduction of NAA and increased mI. We consider our data directly indicative of GM involvement, not detected in previous studies with volumetric analysis, perhaps due to the poor representation of Betz cell in the motor cortex (about 5% of total cells). In conclusion, despite the lack of 100% specificity, we propose that DTI and MRS are useful diagnostic tools for ALS, supplementing functional MRI, by providing a more strict evaluation of the profound state of water diffusion and metabolite composition of neurons.

P119 THRESHOLD TRACKING TRANSCRANIAL MAGNETIC STIMULATION INDICATES CORTICAL HYPEREXCITABILITY IN MOTOR NEURON DISEASE

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Background: Motor neuron disease (MND) is a progressive neurodegenerative disorder of corticomotoneurons and anterior horn cells (AHC), with median survival of two years. Although first described in 1865, site of disease onset remains unknown. A 'dying forward' hypothesis, which proposes that corticomotoneurons cause excitotoxic AHC death, has been proposed as a possible pathophysiological mechanism in MND. This hypothesis may be assessed by using paired-pulse transcranial magnetic stimulation (TMS) techniques to assess cortical excitability. Conventional paired-pulse techniques use a constant stimulus intensity, which is limited due to marked variability of motor evoked potential (MEP) amplitude. Threshold tracking techniques have the potential to overcome this limitation. Although threshold tracking has been extensively utilized to investigate peripheral nerve function, the feasibility of this technique in the investigation of the central nervous system disorders is lacking.

Objectives: The aim of the present study was; 1) to determine the ability of threshold tracking TMS to assess cortical excitability in healthy controls and 2) to apply this technique in patients with MND so as to determine the site of disease onset.

Methods: MEPs were generated in 15 MND patients, and 25 age-matched healthy controls, using a 90 mm circular coil connected to a BiStim 200 dual pulse magnetic stimulator. Surface electrode recordings were obtained from the abductor pollicis brevis muscle. An MEP amplitude of 0.2 mV was tracked by the conditioned test stimulus.

Results: Threshold tracking TMS was successfully undertaken in controls, with two distinct phases of early intracortical inhibition (ECI) evident, peaking at interstimulus intervals (ISI) of 1 ms (7.8% increase) and 3 ms (11.2% increase). ECI continued up to an ISI of 9 ms. Cortical facilitation then followed from an ISI of 10–30 ms. Late intracortical inhibition occurred at ISI of 50–300 ms, peaking at 150 ms (24.2% increase). In 13 MND patients there was significant reduction of ECI, peaking at an ISI of 3 ms (3.1% increase, $p<0.05$). Cortical facilitation developed at ISI of 4 ms and was significantly increased, peaking at ISI of 10 ms (7.6% decrease, $p<0.05$). In two MND patients with advanced disease the motor cortex was inexcitable. There were no significant differences in late inhibition and cortical silent period duration between groups.

Conclusions: Threshold tracking TMS confirms the presence of cortical hyperexcitability in patients with recent onset MND. Simultaneous threshold tracking studies to assess upper and lower motor neuronal function in MND patients may establish the site of disease onset.

P120 COMPARISON OF THE TRIPLE STIMULATION TECHNIQUE AND CONVENTIONAL TMS STUDY FOR THE DETECTION OF UPPER MOTOR NEURON DYSFUNCTION IN ALS

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Background: Diagnosis of ALS needs clinical evidence of both upper motor neuron (UMN) and lower motor neuron (LMN) signs. Evidence of LMN degeneration by clinical and electrophysiological assessment is usually easy. In contrast, evidence of UMN involvement in ALS patients may be elusive, and obscured by the effects of LMN loss. Transcranial magnetic stimulation (TMS) may contribute to the diagnosis of ALS by reflecting UMN dysfunction that is not clinically detectable. Sensitivity of conventional TMS to detect UMN involvement has been widely discussed. The triple stimulation technique (TST), a new collision technique has been suggested to be more sensitive and useful than conventional TMS techniques to detect sub-clinical UMN involvement.

Objectives: To determine, prospectively, the sensitivity of the two different techniques in the assessment of UMN involvement in two groups, definite/probable and suspected/possible ALS patients.

Methods: We studied 66 patients with definite ($n=25$), probable ($n=26$), or suspected/possible ALS ($n=15$) according to El Escorial criteria. All patients were treated by riluzole at the time of the study. Conventional TMS and TST were recorded on the FDI muscle on one side. The following parameters were studied: amplitude ratio (MEP/ CMAP), central motor conduction time (CMCT), excitability threshold (ET), silent period (SP), and TST amplitude ratio.

Results: In the group 1 definite/probable, mean age of patients was 63 ± 11.2 years (range 37–82 years), 34 were males, mean duration of symptoms was 17.9 ± 18.7 months (range 3–96 months), mean score of ALSFRS was 32.2 ± 3.9 (range 21–38). In the group 2 possible/suspected, mean age was 66.2 ± 11.9 years (range 38–74 years), 11 were males, mean duration of symptoms was 19.1 ± 14.5 months (range 1–51 months), mean score of ALSFRS was 35.9 ± 2.7 (range 31–40). For all patients, CMCT was abnormal in 14 cases (21%) and amplitude ratio in 25 (37.9%). ET was decreased in four cases and increased in 16, so abnormalities were found in 30.3%. SP was abnormal in 30 patients (45.5%). Abnormalities for both CMCT and amplitude ratio, the strongest parameters, were found in 53% of cases. At least one abnormality among the four parameters was found in 70% of cases. Though amplitude ratio by TST is highly reproducible (92% in controls), TST amplitude ratio was abnormal in only 47% of the 66 patients.

Conclusions: Conventional TMS is more sensitive than TST in detecting upper motor neuron dysfunction in ALS. Sensitivity can be improved by increasing the number of muscles studied: studying at least three territories increases the probability of detecting corticospinal pathway abnormalities. TST is highly reproducible, but it can be performed only on the distal segments of the upper limbs in clinical practice. This technical limit leads to its low sensitivity.

P121 CEREBRAL HAEMODYNAMIC CHANGES ACCOMPANYING COGNITIVE IMPAIRMENT IN PRIMARY LATERAL SCLEROSIS

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Background: Primary lateral sclerosis (PLS) is a rare neurodegenerative disorder of the upper motor system. This disease may also present clinically significant cognitive impairment that may be associated with cerebral haemodynamic changes.

Objectives: To assess if PLS patients are subject to cognitive decline, as measured by neuropsychological tests (NT), and determine its relationship with alterations in cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT) as measured by computed tomography (CT) Perfusion.

Methods: The PLS patient group consisted of nine males and eight females aged 44 to 72 (mean, 59.11 ± 8.67) years. Data obtained from this group were compared with four male and three female non-PLS controls aged 34 to 63 (mean, 52.86 ± 9.22) years. Patients were then stratified into three groups based on NT scores; those failing 0 test scores ('Normal', $n=4$), 1–3 test scores ('Mild', $n=8$), and 4 or more test scores ('Moderate', $n=6$). On the day of NT, CBF, CBV, and MTT were measured using CT Perfusion. CT images were segmented into the four lobes of the brain and compared across groups and with non-PLS controls. Correlations between CBF, CBV, and MTT and failed NT scores were also analysed.

Results: No significant difference was found between the cognitively 'normal' PLS patients and non-PLS controls for any measured parameter in any region ($p > 0.05$). MTT was significantly increased in the 'moderate' cognitively impaired PLS patients in all regions compared to non-PLS control subjects and cognitively 'normal' patients, with the exception of the temporal lobe in the 'normal' group ($p < 0.05$). CBF was significantly decreased in the 'moderate' group compared to controls for the temporal and occipital region ($p < 0.05$). CBF was negatively correlated with failed NT scores in all regions of the brain ranging from $-0.43 \leqslant r \leqslant -0.68$ ($p < 0.05$). MTT was positively correlated ($0.49 \leqslant r \leqslant 0.65$) with failed NT scores across all regions ($p < 0.05$). CBV was not significantly correlated with failed tests scores and was not significantly different between groups.

Conclusions: The most sensitive haemodynamic marker for changes in cognition was found to be mean transit time. MTT was significantly elevated across all regions in the 'moderate' group when compared to the non-PLS controls. Cognitive decline in PLS patients is associated with the activation of the cerebrovascular reserve, manifested as trends of increasing CBV and decreasing CBF. From these data we can conclude that a subgroup of PLS patients is subject to cognitive decline that is reflected by changes in cerebral haemodynamics as measured by CT Perfusion.

Acknowledgement: This research was funded by ALSA.

P122 DEFICITS OBSERVED IN PATIENTS WITH PRIMARY LATERAL SCLEROSIS ARE ASSOCIATED WITH CHANGES IN CEREBRAL HAEMODYNAMICS

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Background: Primary lateral sclerosis (PLS) is a neurodegenerative disorder involving the upper motor system. Computed tomography (CT) Perfusion can be used to evaluate alterations in cerebral haemodynamic parameters.

Objectives: Our aim was to evaluate whether specific symptoms of this disease could be associated with alterations in CT Perfusion parameters.

Methods: Eighteen patients with PLS, nine males and nine females aged 44 to 72 (mean, 59.11 ± 8.67) years, underwent CT Perfusion scans to determine their cerebral blood flow (CBF) and volume (CBV). Mean transit time (MTT) is a calculated measure: CBV/CBF. CT images were segmented into four lobes and each lobe was further subdivided into grey and white matter (GM and WM, respectively). The Mann-Whitney test was used to determine if haemodynamic parameters differed between groups based on the presence of each symptom at onset and follow-up. SPSS was used to perform statistical analyses.

Results: The presence of bulbar symptoms ($n=12$) was associated with a significantly lower CBV in the GM of the parietal lobe ($p < 0.05$). The presence of cardiovascular disease ($n=7$) was associated with significantly higher CBV in the WM of the temporal and parietal lobe ($p < 0.02$). There was a trend towards significance for onset of stiffness as a presenting symptom ($n=7$) with higher CBV in the grey matter of the basal ganglia, $p=0.085$.

Conclusions: From these data we conclude that certain deficits in PLS patients may be associated with changes in cerebral haemodynamics, which are readily measured using CT Perfusion. Additionally, certain associated diseases such as cardiovascular disease may have an impact or be concomitant with other disease states that affect cerebral haemodynamics.

Acknowledgement: This work is funded by the ALSA.

P123 MOTOR UNIT INVOLVEMENT IN PLS, ALS AND KENNEDY'S DISEASE

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Background: Primary lateral sclerosis (PLS), Kennedy's disease and amyotrophic lateral sclerosis (ALS) are diseases characterized by progressive loss of upper motor neurons (UMN), lower motor neurons (LMN) or both. Clinically, PLS is usually considered as a pure UMN disorder. The rate of motor unit (MU) loss in Kennedy's disease is poorly documented.

Objectives: Aims of this study were to compare the MU involvement in these three diseases, to document possible sub-clinical LMN involvement in PLS and to measure respective rate of MU loss.

Methods: Data were collected from 26 patients with ALS ($n=16$), PLS ($n=5$) and Kennedy's disease ($n=5$). Patients underwent CMAP measurements (tibialis anterior and thenar muscles bilaterally). The CMAP score was calculated by: Σ CMAP area/4. On the less affected side, we assessed MUNE, F-responses and decremental responses (after repetitive nerve stimulation at 3 Hz) from thenar muscles. Thenar MUNE was estimated by using the adapted multiple point stimulation (AMPS) method. The rate of MU loss was calculated by comparing two successive MUNE values. F-index was calculated by: occurrence X maximal peak-to-peak amplitude (32 supramaximal stimuli were applied).

Results:

	PLS	ALS	Kennedy
Age (years)	57 ± 8	57 ± 12	54 ± 7
Disease duration (months)	140 ± 38	35 ± 19	67 ± 56
CMAP score (mV.ms)	34 ± 8 (0/5)	21 ± 13 (8/16)	37 ± 8 (1/5)
Thenar MUNE	68 ± 38 (4/5)	58 ± 65 (12/16)	53 ± 69 (4/5)
Rate of MU loss (%/month)	3.0 ± 2.1	7.2 ± 7.7	0.5 ± 0.1
Decrement (%)	4.8 ± 0.8 (0/5)	12.2 ± 7.6 (8/16)	6.0 ± 2.1 (0/5)
F-index	245 ± 104	70 ± 93	52 ± 22

(number of values beyond limits of normal)

Conclusions: Thenar MUNE was reduced in the three groups without significant difference between them. Rate of MU loss was high in ALS (7.2% per month), low in Kennedy's disease (0.5% per month) and intermediate in PLS (3.0% per month). Thenar MUNE was more sensitive than CMAP to detect MU involvement, particularly in chronic diseases. In fact, in PLS and Kennedy's disease, CMAP scores remained within normal limits in 9/10 patients, whereas thenar MUNE was decreased in 8/10 patients. In ALS patients, disease duration at baseline was shorter than in other patients and there were more cases with decremental responses (greater than 10% in 8/16 patients). These findings were consistent with a subacute course. In PLS, we found a subclinical MU involvement in 4/5 patients and an increase in the F-index probably related to the predominant UMN involvement. In Kennedy's disease, decreased thenar MUNE with a very slow MUNE decline suggested a MU loss starting early in life and with long-lasting subclinical course.

P124 EMG SURFACE INTERFERENCE PATTERN-INDEX MEASUREMENT IN ALS

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Background: EMG surface interference pattern (SIP) contains information on motor unit (MU) number and size that can be useful in assessing neurogenic disorders such as ALS.

Objectives: We have developed a new measurement of the SIP called SIP-Index that is derived from the compound muscle action potential (CMAP) measurement.

Methods: After obtaining the CMAP, the same surface electrodes were used to record the SIP at various force levels ranging from slight to maximal effort. A plot of SIP-Index versus SIP area was then constructed. An area within this plot, called the 'normal cloud', contained all expected data points for a normal muscle. We recorded SIP (10 epochs per study) and the CMAP from the hypothenar muscle of 28 healthy subjects to develop the normal cloud. Ten patients with definite ALS were tested.

Results: In ALS patients, data points fell on the lower side and outside of the normal cloud reflecting MU loss. This pattern was seen even when the CMAPs had normal amplitudes. This reflected increased MU size. With significant MU loss, data points were further away from the normal cloud and closer to the abscissa of the plot. During visual assessment of the SIP, high amplitude, fast-firing MUs were easy to recognize. SIP can be recorded fairly quickly in less than 5 min.

Conclusions: This method can be used as a 'quick' screen to assess MU number and size abnormalities in patients during the routine motor conduction studies.

P125 CORRELATION BETWEEN DISTAL MOTOR LATENCY AND COMPOUND MUSCLE ACTION POTENTIAL IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder, characterized by a selective progressive degeneration of the motor system. Electromyography is essential for the diagnosis of ALS.

Objectives: The measurement of motor conduction of peripheral nerves is of major importance to recognize other possible causes of progressive muscle wasting. However, there are also pathological changes in nerve conduction studies in ALS patients.

Methods: In this prospective study we analysed the values of distal motor latency (DML), compound muscle action potential (CMAP) and motor nerve conduction velocity (MNCV) in 95 patients with definite ALS.

Results: We found slight slowing of MNCV and moderate to strong reduction of CMAP and a prolongation of DML. We found no significant correlation between MNCV and CMAP.

Conclusions: The main finding of the present work was the negative correlation between DML and CMAP. It is interpreted as a very distal axonal damage as the main reason for prolongation of DML in ALS patients.

P126 PROGNOSTIC VALUE OF AMPS METHOD IN ALS PATIENTS

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Background: The adapted multiple point stimulation (AMPS) method is a manual motor unit number estimate (MUNE) technique which is reliable, non-invasive, comfortable and easily performed in patients and on any EMG device. Previous studies have demonstrated the high sensitivity of MUNE techniques to detect and quantify motor unit (MU) loss in amyotrophic lateral sclerosis (ALS) patients and changes over time.

Objectives: The goal of this study was to determine the AMPS efficiency to distinguish ALS patients according to a slow or rapid progression and in predicting prognosis.

Methods: Data were collected from 16 patients with ALS, age ranging from 36 to 74 (mean 60 ± 10) years. Patients died after a mean total disease duration (TDD) of 38 ± 20 months (range 8–72; nine patients died before three years TDD and seven patients later). AMPS was applied at least twice in each patient at 3–4 month intervals. Incremental stimulation (50 μs stimulation duration, weak intensity gradually increased by increments of 0.1–0.5 mA) was used to allow sequential activation of individual motor axons. Incremental stimulation was administered at distinct points along the median nerve. At each stimulation point, two or three motor unit potentials (SMUP), free of alternation, were successively evoked. By dividing the maximum CMAP size by the average SMUP size, a MUNE was obtained. Survival probability was calculated by Kaplan-Meier method.

Results: MUNE at baseline (cross-sectional study) was correlated with the time to death in patients with TDD longer than three years only ($r=0.80$). The rate of MU loss (longitudinal study), calculated by comparing the last and initial thenar MUNE measurements, was negatively correlated with TDD ($r=-0.83$). Thenar MUNE decline was less ($n=8$) or more ($n=8$) than 30% over four months and less ($n=6$) or more ($n=6$) than 40% over eight months. The best survival probability was observed in patients with the lower MUNE decline. In these patients, after the first four months, survival probability was 50% at month 18 (versus 0% in patients with MUNE decline more than 30%) and after the first eight months, survival probability was 83% at month 9 (versus 0% in patients with MUNE decline more than 40%).

Conclusions: This cross-sectional study suggested that thenar MUNE might be a survival indicator. In fact, in patients with a slow rate of progression (TDD > 3 years) the higher the thenar MUNE at baseline, the longer time to death. This was confirmed by the longitudinal study. There was a significant negative correlation between the rate of MU loss, estimated by AMPS, and TDD. Survival curves indicated that the prognostic value of AMPS was better after a MUNE evaluation over eight months than over four months.

P127 DIFFERENTIAL MARKERS OF CHRONIC PARTIAL DENERVATION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS AND BENIGN MOTOR NEURON DISORDERS

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Background: Previous needle electromyography (EMG) studies suggest that duration of motor unit potentials (MUPs) are increased in affected muscles in amyotrophic lateral sclerosis (ALS) and benign motor neuron diseases (BMNDs) and increase with the course of the disease over six months follow-up.

Objectives: We verified feasibility of short interval follow-up needle EMG in ALS and BMND for differential diagnosis.

Methods: We studied 25 patients with definite ALS, 10 patients with BMNDs (spinal amyotrophies) and 10 healthy volunteers. Needle EMG was performed in trapezius, adductor pollicis brevis and gastrocnemius lateralis muscles three times with two-month intervals.

Results: Test-retest correlation in healthy volunteers was $\kappa=0.85$–0.9, $p<0.05$; in muscles with MUPs duration lower than -20%, duration and amplitude correlated negatively ($r=-0.7$; $p=0.02$) and where it was over -20% correlation was positive ($r=0.4$; $p=0.03$). This suggested reliability of data. All studied muscles in BMNDs had no paresis and higher MUPs duration (mean 27.9, range 24.4–32.5; and mean -6.4%, range -19.7 to -4.4; $p<0.0001$), than 75 muscles in the ALS group (4–5 degrees of paresis by MRC scale). MUPs duration increased in follow-up study in muscles close (+6.9%) and far from site of onset (+18.3%), but

decreased within site of onset (−6.5%). This was not observed in BMNDs.

Conclusions: MUPs duration over +20% in muscles with no paresis is a differential BMNDs marker, but not for ALS. In ALS high MUPs duration accompanies severe paresis. In follow-up study in ALS, but not BMNDs, MUPs duration may decrease in muscles at the site of onset.

P128 COMPARISON OF A COMPOUND MUSCLE ACTION POTENTIAL MEGASCORE (CMAPM) AND FORCED VITAL CAPACITY (FVC) TO FOLLOW DISEASE PROGRESSION IN ALS

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Background: Objective markers for disease progression are urgently needed. CMAP measures of various muscles have been used and found to be sensitive to change. This also applies for the forced vital capacity (FVC) which is a recommended and established measure in ALS clinical trials. However, it is not clear if any of these measures is superior when applied in a multi-centre randomized controlled clinical trial.

Objectives: To evaluate whether FVC or a CMAPM is a better marker of disease progression.

Methods: Thirty-one ALS patients with a mean age of 59 (32–75) years were enrolled in a randomized study (Novartis TCH 346A) at two sites. Patients underwent ALSFRS-R scoring, CMAP (ABP, ADM, EDB, AH bilaterally) and FVC measurements over a 1-year period at regular 8–12 weekly intervals. The CMAP megascore (CMAPM) was calculated by: CMAP amplitudes (ABP, ADM, EDB, AH bilaterally)/8. Measures at the beginning and during follow- up were compared using the paired *t*-test.

Results: At study entry mean FVC was 99.8 ± 15.0. Mean CMAPM was 5.23 ± 2.7 and mean ALSFRSR 41.4 ± 4.0. After 12 months FVC had dropped to 57.6 ± 15.8, CMAPM to 2.3 ± 2.7 and ALS-FRSR to 32.9 ± 7.8. Changes became significant for FVC and CMAPM at week 24 ($p < 0.003$, paired *t*-test) and ALS-FRSR at week 16 ($p < 0.006$, paired *t*-test). Slope of decline was similar for FVC, CMAPM and ALSFRS. The correlation between ALS-FRSR and FVC ($r = 0.48$; $p < 1.5 \times 10^{-7}$) was slightly greater than between ALS-FRSR and CMAPM ($r = 0.41$; $p < 1.0 \times 10^{-6}$). In six patients FVC could not be reliably obtained through the entire observation period due to leakage of the mouthpiece, but CMAPM measurements could be performed at all times.

Conclusion: FVC, CMAPM and ALSFRSR show a stable decline over a 1-year period and are sensitive markers of disease progression. Both FVC and CMAPM exhibit a significant correlation with the ALSFRS-R. However, complete measurements during the entire observation period could be only obtained for CMAPM measures. This suggests that in addition to FVC, neurophysiological markers should be used in multi-centre randomized clinical trials.

P129 USE OF PERCENT OF PREDICTED NORMAL STRENGTH VERSUS MEGASCORE SLOPES TO MEASURE DISEASE PROGRESSION IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Over the past two decades, use of maximal voluntary isometric contraction (MVIC) has become a standard outcome measure in ALS clinical trials. Megascores are calculated by converting raw MVIC values to z-scores for each muscle group based on a population mean and standard deviation derived from a natural history ALS databank. The arm megascore is the mean of 10 individual arm z-scores. Disease progression is then expressed as the slope of the megascore over time. An alternative method for summarizing MVIC data involves converting raw MVIC values to percent of predicted normal (PPN) using regression equations derived from a databank of 493 healthy adults. The regression equations use each individual's age, gender, height and weight to produce a predicted MVIC value for each muscle group. The ratio of the raw score and the predicted score multiplied by 100 yields the PPN score. The average PPN arm score is the mean of the 10 individual arm PPN scores. Disease progression is then expressed as the change in PPN over time.

Objectives: To compare two methods that use MVIC data to describe disease progression: 1) megascore slopes, and 2) change of percent of predicted normal (PPN) over time.

Methods: Data from 10 males and 10 females in an ALS natural history databank containing monthly MVIC tests over a 1-year period were randomly selected for analysis. The disease progression was calculated using two methods: 1) the slope of the arm megascore, and 2) the change of PPN arm score per year.

Results: Although the correlation between megaslope and PPN slope was high (0.84) for the combined group of 20, the two methods revealed significant differences in subgroup analysis comparing males and females. The average change in the arm PPN over the year was lower in males than females (28% vs. 35%), yet the arm megascore slope was greater in the subgroup of males versus the subgroup of females (mean arm megascore slope was 1.4 for males vs. 0.9 for females).

Conclusions: Megascores control for differences between large and small muscles. However, megascores do not account for personal factors that determine normal strength. For example, MVIC of a young male should be more than twice as high as an elderly female, assuming they both have normal strength. By controlling for differences in age, height, weight, and gender, PPN allows a fair comparison between subjects who have large differences in their expected strength due to these factors. Therefore, use of PPN to summarize MVIC data may offer a more accurate comparison between subjects, by neutralizing the effects of gender, age and size.

P130 CAN WE PREDICT DISEASE PROGRESSION IN ALS?

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Background: Survival has been a major study endpoint in ALS clinical trials, and the prognostic factors influencing survival have been well defined. As an outcome measure in clinical trials, survival mandates prolonged trials with large numbers of patients. Using disease progression as an endpoint would reduce the need for large patient cohorts and reduce the length of the study. However, to reduce the heterogeneity of the population enrolled, and to improve baseline stratification, a greater understanding of the relevant predictors of disease progression would be required; and at present such data are lacking.

Objectives: In order to determine the predictors of disease progression we performed a historical cohort study of ALS patients referred to our ALS Clinic over the last 20 years.

Methods: In a group of 832 patients with the diagnosis of definite or probable ALS the effects of individual prognostic factors on disease progression were assessed with the Kaplan-Meier life-table method. Disease progression was defined as a time to 20-point change in Appel score (AALSS), which reflects a change in patient's lifestyle and clinical status. In addition, the prognostic value of each factor was estimated using both univariate and multivariate Cox proportional hazard analyses.

Results: The median time to a 20-AALSS-point change in our patient population was nine months. Age, site of symptom onset, diagnostic delay, total AALSS at first exam and AALSS preslope (rate of disease progression between first symptom and first examination) have been revealed as significant and independent covariates of disease progression in our population. Forced vital capacity (FVC) has been shown to be a significant covariate of disease progression in both univariate and multivariate Cox model after adjustment for age, sex and site of onset but was eliminated as an independent predictor in the final statistical model.

Conclusions: Our results support the use of a 20-point change in Appel score as a reliable study endpoint, which can be achieved within a 1-year clinical trial. Moreover, the identification of predictors of disease progression may facilitate better design of therapeutic trials, permitting the use of disease progression as a primary endpoint and improving baseline stratification of patient populations. In addition, defining the prognostic factors that influence disease progression may help improve patient management and care.

P131 ALS-FRS AND APPEL ALS SCORES: DISCORDANCE IN ADVANCED STAGES OF DISEASE

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Background: The ALS functional rating scale (ALS-FRS) score is a validated questionnaire used to measure ALS progression. It includes no objective measurement of strength or breathing capacity. The Appel ALS (AALS) score is also a validated measure of ALS progression, consisting of 5 subscores measuring swallowing, respiratory function, muscle strength, and upper & lower extremity function.

Objectives: To determine if the ALS-FRS & AALS scores correlate at different stages of the ALS disease.

Methods: We examined the ALS-FRS and AALS scores for 32 ALS patients at their visits over 10 months, a total of 68 AALS and ALS-FRS score pairings, with an average of two visits per patient. A Spearman's rho correlation analysis and a multiple regression analysis were conducted on the AALS total score to determine their relationship to the ALS-FRS total score.

Results: ALS-FRS and AALS scores significantly correlated when the complete range of scores for each was considered ($p < 0.01$). However, when analyzed for Appel score-defined life-altering changes as measured by a 20-point change in the AALS scale score (40–60, 60–80, 80–100, 100–120, >120), there was significant correlation ($p = 0.008$) only in the 40–60 range, the initial stage of the disease. In all other ranges, the AALS score and ALS-FRS correlation did not reach significance.

Conclusions: Our findings suggest that objective measurements from AALS and questionnaire based ALS-FRS scores may not correlate as the disease progresses, especially in the advanced stages. Our results suggest that further studies of validating questionnaire-based measurements against multiple system measurements at different stages of the disease are needed before functional questionnaires are accepted as sufficient measurement tools for therapeutic trials in ALS.

P132 NON-LINEAR BEHAVIOUR OF THE ALS-FRS IN ALS PATIENTS TOWARD THE END OF LIFE

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Background: The ALS-FRS disability scale is usually regarded as behaving linearly throughout the disease. This is important in the evaluation of new drug treatments and in the planning of clinical trials. Existing data on linearity of ALS-FRS were obtained mainly from patients with mild to moderate disease participating in clinical trials. There are no available data about the sequential changes in ALS-FRS over time in the last part of patients' lives.

Objectives: To compare the slope of deterioration of ALS-FRS in ALS patients during mild to moderate stages of disease and toward end of life in order to determine if the slope changes over time and if this change is predictive regarding survival.

Methods: Among 295 patients with ALS followed up in our clinic during recent years, 60 had at least four visits with documented ALS-FRS and time of death. For each of these patients we calculated the maximal FRS slope, the critical time (time elapsed between the point of maximal slope and death) and the critical FRS (FRS value at which the maximal slope occurred).

Results: Included patients had 4–16 FRS determinations (mean 5.6) over a period of 16–137 months (mean 30). Their critical time occurred 2–74 months before death (mean 16). The critical FRS had a broad range of values of 5–38, with a mean of 25. The critical time was negatively correlated to the maximal slope ($p=0.027$). Stratification of maximal slope values by critical time showed that patients with steeper maximal slopes had a shorter critical time (less than one year) compared to those with lower slopes, compatible with more aggressive disease.

Conclusions: The ALS-FRS behaves linearly for the greater part of follow-up in patients with ALS, but changes its curve late in the disease. Changes in the slope of deterioration of FRS could serve as an additional prognostic factor for survival.

P133 THE PERFORMANCE OF AN INSTRUMENT TO MEASURE CHANGE IN DYSPNEA IN PATIENTS WITH ALS

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Background: Respiratory complications are the most common cause of mortality in patients with ALS. Limitations in activity from limb muscle weakness can make assessing dyspnea difficult. It is important to be able to accurately measure dyspnea over time for assessment and treatment of patients and as an outcome in clinical studies.

Objectives: This study evaluated the Baseline Dyspnea Index (BDI) and Transitional Dyspnea Index (TDI), a measure of change from baseline, in subjects with ALS.

Methods: Subjects were enrolled in a randomized, controlled trial of high frequency chest wall oscillation (HFCWO) and had El Escorial probable or definite ALS, an ALS Functional Rating Scale respiratory subscale (ALSFRS-RS) $\leqslant 11$ and >5, and FVC $\geq 40\%$ predicted. Pulmonary function tests, ALSFRS, and dyspnea scales were measured at baseline, four weeks, and 12 weeks after enrolment. The BDI and TDI consist of three categories: functional impairment, magnitude of task, and magnitude of effort. Each category of BDI is graded from 0 (severe) to 4 (unimpaired). In the TDI, changes in dyspnea are graded from -3 (major deterioration) to +3 (major improvement). Intraclass correlation coefficients (ICC) were calculated to determine reproducibility of the BDI and TDI within patients. ICCs were calculated for 11 subjects in the BDI and 10 in the TDI. Differences in BDI-TDI scores were compared by χ^2. TDI scores were compared with changes in FVC, ALSFRS-RS, and a visual analog scale (VAS) of breathlessness using Spearman rank coefficients and *t*-tests.

Results: Forty-six subjects were enrolled and 35 returned for the 12-week follow-up. Twenty-two subjects were assigned to HFCWO. Although there was a trend for more deterioration in dyspnea in the control group compared to HFCWO, this was not significant and data are presented for the two groups combined. Forty-six percent were male, 74% were Caucasian, and 50% had college degrees. The mean FVC was $66.3 \pm 14\%$, 50% used BiPAP, and the mean ALSFRS-RS was 9.1 ± 1.9. The ICC for each category of the BDI and TDI was ≥ 0.98 indicating near perfect reproducibility. By BDI score at entry, 15 subjects (32.6%) had moderate to severe functional impairment due to dyspnea, 25 (54.3%) reported moderate tasks or less resulted in

dyspnea, and 25 subjects reported that moderate effort or less resulted in dyspnea. At four weeks, 15 (38.5%) patients reported deterioration in at least one TDI measure and at 12 weeks, 19 (54.3%) had deterioration in at least one TDI measure. Only 5/17 (29.4%) of patients had any decline in the respiratory subscale of the ALSFRS. On a 10-point VAS (0=no breathlessness and 10=maximal breathlessness), the mean rating at baseline was 2.8 and actually decreased at week 12 to 2.4. Deterioration in TDI was significantly associated with decline in FVC and was significantly correlated with worsening breathlessness and ALSFRS-RS.

Conclusions: The BDI-TDI is highly reproducible in patients with ALS, is sensitive to changes in dyspnea in as little as one month and correlates with other measures of dyspnea. The BDI-TDI has potential importance for clinical management of patients and as an outcome in clinical trials.

Acknowledgement: Support for this study was provided by Hill-Rom Inc.

P134 INTRODUCTION OF ELECTRONIC DATA CAPTURE AND DATA MANAGEMENT TECHNOLOGY TO INVESTIGATOR-INITIATED CLINICAL TRIALS AND BIOMARKER STUDIES IN AN ACADEMIC ENVIRONMENT

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Background: Out of thousands of active clinical trials at academic institutions approximately one-third are investigator-initiated studies. The efficiency and regulatory compliance of conducting such trials are as important as the quality of the results. Running in-house coordination centre and data management operation for more than one multi-centre trial is an extremely aggressive target and difficult to maintain using paper-based processes for managing clinical trials. PharmaENGINE℠ Electronic Data Capture (EDC) and Data Management (DM) platform deployed by Neurology Clinical Trials Unit (NCTU) enables investigators and coordinators to directly record trial data over the internet, using electronic Case Report Forms (eCRFs) instead of the traditional paper CRFs. The system validates the data at the point of entry, raises alert queries and utilizes a central server. Data validation at the input stage ensures accrual of fewer invalid subjects. The availability of real time information also gives trial sponsors, PIs and project managers an early warning of issues in the trials (e.g. subject recruitment, adverse events). Information is loaded into the clinical trials database in real time, making subject data instantly available to data managers, coordinators and management, thereby overcoming the traditional delay period. Data validation at the point of entry and rapid query response improves data quality. The intuitiveness and efficiency of user interface and data clarification workflow plays a significant role in speeding up clinical trial processes and reducing overall costs. EDC and DM systems also improve time to database lock and data analysis, as availability of accurate information allows biostatisticians to undertake statistical analysis of the trial within days, not months, after the last patient completes the study. The system also readily provides interim analysis of data during the trial.

Objectives: A comparison of data management models between two clinical studies conducted by the NCTU was performed.

Methods: The first study, a 25-center 300-subjcect clinical trial of celebrex in ALS was conducted over a 3-year period using a paper-based model. In another study, Clinical Trial of Coenzyme Q10 in Subjects with ALS (MDA), a 4-center, 30-subject trial utilized the EDC system.

Results: More than 2200 Data Clarification Forms were written and faxed to the sites in the first study, taking more than four months to receive site responses and entering and cleaning the data to lock the database. In contrast, within three weeks of the second study's last subject's visit, the database was locked and data analyzed.

Conclusions: The significant reduction in queries, the corresponding cost reduction in dealing with the remaining queries resulting in faster database lock, and data analysis makes the EDC and DM model the only viable alternative for academic Coordination and DM centers. To ensure a successful EDC deployment four key components (technology, logistics, process change and organizational change) need to be addressed.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 138–145

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THEME 7 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P135 EFFECT OF SWALLOWING PROBLEMS ON MOOD AND QUALITY OF LIFE IN MOTOR NEURON DISEASE

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Background: The experience of motor neuron disease is associated with anxiety and depression but evidence suggests that disturbed mood does not correlate closely with severity of physical impairment in disabled people. Swallowing problems may be expected to contribute to mood and quality of life (QOL), and gastrostomy feeding is sometimes justified on these grounds. This paper reports early results from an ongoing prospective study of swallowing disorders and nutritional management in MND.

Objectives: To explore the relationship between swallowing severity and anxiety, depression and quality of life in MND.

Methods: Patients with MND and their carers were followed-up at 3–4-monthly intervals using the following outcome measures: total disease severity and bulbar severity (ALS functional rating scale), swallowing symptom severity (SWAL-QOL Outcomes Tool for Oropharyngeal Dysphagia), anxiety and depression (Hospital Anxiety and Depression scale) and quality of life (single-item scale, physical well-being, psychological and existential sub-measures of the McGill Quality of Life questionnaire).

Results: Thirty-two patients and 26 carers (mean age 62.3 and 62.4 years, respectively) were recruited. Sixteen patients commenced gastrostomy feeding during the study. Thirty-one percent of patients and 42% of carers were found to have abnormal anxiety levels at entry to the study, while 22% of patients and 19% of carers scored abnormal for depression. At the visit prior to gastrostomy, the values for anxiety were 44% of patients and 62% of carers and those for depression were 31% and 31%, respectively. Paired *t*-test showed no significant difference in anxiety or depression between patients and carers. No correlations were found between anxiety or depression and either bulbar severity or swallowing symptom severity in patients or carers at either time point. There was also no correlation with total disease severity. For quality of life (patients only), significant correlations were found between swallowing severity score and the McGill Physical well-being score ($r=0.388$, $p=0.028$) at entry, and with the single-item-scale ($r=0.512$, $p=0.044$) at pre-gastrostomy. No correlations were found between the bulbar score and quality of life.

Discussion: These data showed no consistent relationship between mood and swallowing problems, although certain aspects of quality of life did appear to be influenced by swallowing symptoms.

Conclusions: Our findings suggest that the sources of anxiety and depression are complex. It is unlikely that gastrostomy alone would have a major impact on anxiety or depression in a patient with MND.

P136 QUANTITATIVE SENSORY TESTING IN THE ASSESSMENT OF LARYNGEAL SENSATION IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS

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Background: ALS is a progressive motor neuron disease of unknown etiology. Mortality in the population is frequently due to aspiration pneumonia. Although typically considered to be a disorder limited to motor neuron involvement, some investigators have indicated that decreased sensory function in ALS patients additionally contributes to the disease process.

Objective: The objective of this study was to evaluate laryngeal sensation in the ALS population in order to quantify the range of sensory deficits and correlate any abnormalities with demographic data to determine which patients are at risk for having sensory deficits.

Methods: We examined the sensation of the larynx in 27 patients with ALS to determine whether a sensory deficit was present. Following the completion of a dysphagia questionnaire and medical history, patients underwent flexible endoscopic evaluation of swallowing with sensory testing (FEESST) to evaluate sensory function. Threshold values were determined and recorded for initiation of the adductor reflex.

Results: The results of the sensory and swallowing function assessments performed on 27 patients demonstrate abnormal sensation in 44% of the rested population. Asymmetrical findings were noted in 66% of those patients. There was no correlation noted between the presence of sensory deficits and the severity or duration of the disease.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045050

Conclusion: Progressive dysphagia in the ALS population has typically been attributed to muscle weakness. This study points to the presence of sensory deficits in the larynx, which can further affect proper swallowing function.

P137 NUTRITIONAL ASSESSMENT IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a degenerative disease of the nervous system that affects motor neurons; it is progressive and irreversible. Nutrition is an independent prognostic factor for survival: therefore the assessment of nutritional status is important because of the impact of suboptimal nutrition on function, quality of life and life expectancy.

Objectives: To evaluate the patients' nutritional state and nutritional problems.

Methods: We performed a nutritional assessment in all patients diagnosed with ALS evaluated in our centre by a multidisciplinary team, as from April 2004. Nutritional evaluation included: questionnaire about patients' ability to eat, chew and swallow; history of body weight and weight loss; dietary habits and food intake; anthropometry; height, weight and body mass index (BMI=weight [kg]/height $[m]^2$), triceps skinfold thickness, mid-upper arm circumference and mid-upper arm muscle area (MAMA). Nutrition counselling and feeding strategies were provided to all patients and carers.

Results: We observed 72 patients (38 males, 34 females; mean age 52.67 ± 13.62 years, range 20–79 years), of whom 25.0% had bulbar clinical presentation, at 30.12 ± 20.35 months from diagnosis (range 2–82 months). We observed ability to autonomously eat in 45.8%, chewing difficulty in 27.5%, dysphagia in 18.1% (mainly for liquid foods) and occasional swallowing difficulties in 43.0% of cases; 4.2% had percutaneous endoscopy gastrostomy (PEG). Mean BMI was 23.85 ± 4.43 (range 14.5–34.9), <18.5 in 16.7%, 18.5–24.9 in 48.6%, $\geqslant 25$ in 34.7%; 62.5% showed weight loss compared to usual body weight. MAMA was below 5° percentile in 51.9% of cases. Information about correct nutrition was provided to all patients able to eat by mouth; moreover in 26.4% a recommendation was made to modify the consistency of foods, 20.8% were educated in simple behavioural feeding techniques and 15.3% were referred to a speech and language therapist.

Conclusions: The symptoms and progression of ALS can affect the patient's nutritional status, limiting feeding ability (progressively weakening muscles, chewing and swallowing difficulties, dysphagia), leading to weight loss and malnutrition. Individualized nutritional assessment and monitoring should be a part of the treatment in ALS patients to develop an adequate nutritional support.

P138 NUTRITIONAL STATE EVOLUTION AND ALIMENTARY CONSUMPTION OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS: RANDOMIZED STUDY

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Introduction: Amyotrophic lateral sclerosis (ALS) is an evolutionary disease, irreversible and incurable. Observable symptoms include a decrease of vital capacity, loss of corporal weight, an increase in nutritional needs and dysphagia. Some studies suggest that the indication of alternative nutritional support in patients with ALS can improve quality of life and minimize weight loss during the evolution of the disease.

Objective: To evaluate the nutritional state and the alimentary consumption of ALS patients and compare nutritional with alternative nutritional support in relation to patients without alternative nutritional support.

Methods: ALS patients were selected (15 patients with percutaneous endoscopic gastrostomy (PEG) in agreement with criteria proposed by Silani (2000) and 15 patients without PEG). The study was six months long and in this period nutritional evaluations were accomplished as anthropometric measures (corporal weight, arm circumference, arm muscular circumference and triceps skinfold) and trimestrial alimentary consumption using a 24 h alimentary record. The classification of the Nutritional State was based on the patterns of Frisancho (1990) for Arm's Circumference, Arm's Muscular Circumference and triceps skinfold; and the weight classification was obtained using the Body Mass Index (BMI) proposed by the World Health Organization (1998). The alimentary consumption analysis, in relation to calorie and protein intake, was accomplished by the CIS/EPM software.

Results and discussion: The patients' age varied from 31 to 74 years, with median of 53.3 years; 19 (63.3%) were male and 11 (36.7%) female. Analysis of the anthropometric measures indicated that the patients with PEG presented reduction in all measures (corporal weight, arm circumference, arm muscular circumference and triceps skinfold) compared to the group without PEG, where the measures remained unchanged. Protein and calorie ingestion was greater in the patients with PEG compared with the group without PEG. Despite the appropriate protein

and calorie ingestion in the group with PEG, a nutritional state deterioration was observed. Those patients accomplished the PEG procedure tardily, as they had already presented with a 50% reduction in forced vital capacity in 50% of the foreseen, which in most cases is the decisive factor for the increase of nutritional demand, in addition to characterizing the advanced stages of the disease. The patients without PEG received nutritional orientation, mainly about the use of alimentary supplements, which possibly favoured the nutritional state maintenance. In conclusion, early indication of an alternative nutritional support in patients with ALS can attenuate the weight loss presented during the disease evolution.

P139 NUTRITIONAL STATUS AND BIOELECTRICAL IMPEDANCE ANALYSIS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Nutritional deficits are an independent predictor of life expectancy for patients suffering from amyotrophic lateral sclerosis (ALS). This is caused by development of bulbar paralysis with difficulty in swallowing, hypermetabolism due to an increased effort to mechanically overcome pulmonary failure, and an intrinsic increase of energy consumption of unknown cause. The decline in muscle bulk is the basis of potential catabolism, and in addition many patients experience decreased gastrointestinal motility and also dehydration. Novel aspects of the disease process were recently described in animal experiments, in particular alterations of energy consumption which showed the importance of a sufficient calorie intake in experimental models. ALS is a prototype neurodegenerative disease in which the need for nutritional intervention is obvious, but currently it is not known how much nutritional intervention is needed.

Methods and results: We collected data on the nutritional status of 94 early ALS patients (mean age at disease onset 53.3 ± 13.6 years; 30 females, 64 males) and performed bioelectrical impedance analysis (BIA) in the course of disease of 47 patients. During initial stages, there is no evidence for deficits of vitamins (B12, B6, B1, vitamin E and C). Furthermore, no abnormalities in body weight, body mass index, methylmalonic acid, homocysteine, haematocrit, creatinine, ketone bodies, parameters of lipid metabolism, albumin and prealbumin and fasting blood glucose were found. Only serum levels of CK were elevated. In contrast, performance of BIA showed distinct significance abnormalities in estimation of free fat mass and phase angle (PA) regardless of the underlying disease subtype. This indicates that an alteration in body composition of unknown mechanism is present in early ALS stages.

Conclusions: Our data on the nutritional status of ALS patients in early stages do not show changes of weight, vitamin deficiency or catabolism as measurable by laboratory tests or abnormalities in lipid metabolism, but there are changes of parameters obtained by BIA. BIA is supposed to be a valid test of the nutritional status in the course of the disease. However, for the future it is necessary to obtain follow-up BIA data in the course of ALS to assess its value for monitoring nutritional status, as metabolic and caloric requirements of ALS patients, especially in the late phase, are not well understood at present.

P140 HYPERMETABOLISM IN ALS: CORRELATIONS WITH CLINICAL PARAMETERS AND SURVIVAL

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Background: ALS is a neurodegenerative disorder characterized by upper and lower motor neuron degenerations leading to an extensive paralysis with muscle atrophy. Resting energy expenditure (REE) is linked to fat free mass (FFM), age and sex. Despite a reduction in FFM, a hypermetabolism has been reported with an average of 10% in ALS patients compared with a healthy population (1).

Objectives: To confirm the level of hypermetabolism in a larger group of ALS patients, to study variations over time and correlations with clinical and paraclinical parameters and survival.

Patients and methods: One hundred and sixty-eight patients with probable or definite ALS were prospectively enrolled. REE level was determined by using indirect calorimetry. Difference with calculated value given by Harris and Benedickt equations determined a ΔREE. FFM was given by bioimpedance dual frequency (5–100 kHz). Phase angle, which is a physical parameter of cellular integrity was analysed in BIA evaluation. At each consultation, body mass index (BMI) was calculated. Neurological assessment was performed using MMT and ALSFRS scores. Forced vital capacity was evaluated by spirometry. Survival was analysed from first symptoms of ALS and from the first evaluation at T1 (mean delay from first symptoms: 556 ± 524 days). Correlations with clinical and paraclinical parameters were studied on REE and ΔREE levels. Survival was analysed in univariate and multivariate analyses after adjusting for factors with a p value between 0.25 and 0.05.

Results: At T1, REE was significantly increased by an average of 14% compared with calculated value; 62.3% of ALS patients were considered as hypermetabolic (REE measured exceeding 110% of the calculated value). In univariate analysis, REE was correlated with age, sex,

clinical form at onset, presence of a denutrition determined on BMI, weight, FFM, phase angle and ALS-FRS. In multivariate analysis, REE was linked to age and FFM. ΔREE was correlated with sex, phase angle and MMT in univariate analysis. In multivariate analysis, age and sex remained significantly correlated. Over time, REE levels remained higher than calculated values with a trend to decrease at proximity of death, whereas FFM remained stable near the period of death. Survival from first symptoms was linked to age and clinical form at onset. From T1, survival in univariate analysis was linked to MMT, ALSFRS, FVC, REE and phase angle. In multivariate analysis, age, FVC and BMI were correlated.

Conclusion: We confirmed the existence of a hypermetabolic state in the ALS population. Hypermetabolism remains stable over time except at the proximity of death. It depends mainly on age, sex and FFM. REE is a prognostic factor for survival in univariate analysis. Its cause remains poorly understood. Some hypotheses including cytokine secretion, mitochondrial disturbances or sympathetic dysfunction have to be addressed.

Keywords: Hypermetabolism, Survival, Nutrition

References

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P141 PERCUTANEOUS DILATATIONAL TRACHEOSTOMY USING UNCUFFED SMALL CANNULA FOR PALLIATIVE CARE IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Patients with terminal amyotrophic lateral sclerosis (ALS), particularly the bulbar type, often have a high risk of upper airway obstruction due to aspiration of secretions, accompanied by the fear of ''choking to death''. This problem occurs suddenly and is not reduced by the administration of opioids.

Objectives: Intra-tracheal suction can be approached via the nose or mouth, but this unpleasant and blind technique is often unsuccessful. Frequent suction may exhaust patients. Percutaneous dilatational tracheostomy (PDT) using the Seldinger technique has a low incidence of complication compared with surgical tracheostomy and provides an access to the trachea for suction. The purpose of this study was to assess the safety and efficacy of PDT for palliative care in patients with ALS.

Methods: Three ALS patients in terminal phase received PDT. These patients were extremely distressed by the accumulated secretion and declined ventilation support via tracheostomy. PDT was performed using a Mini-Track II minitracheostomy kit or Melker emergency cricothyrotomy catheter set. Placement of a small uncuffed cannula (inner diameter: 4 or 6 mm) was achieved at the cricothyroid membrane or between the cricoid and the first tracheal ring.

Results: In all cases, PDT was easily and safely performed without any trouble. There were no complications such as pharyngotracheal aspiration caused by the cannula and cricoid stenosis. Two patients were under local anesthesia, and the other was under light inhalation anesthesia during the PDT procedure. Intra-tracheal suction was performed immediately when patients noted distress due to airway obstruction by secretion. Speech and swallowing functions were not affected by the cannula. Two patients were able to go home soon after receiving the procedure. All patients died peacefully without ventilation support. The calmness of their final days was maintained by intermittent suction via the cannula and administration of opioids. The cannula was retained for periods ranging from 2 to 3.5 months until death.

Discussion and conclusions: Standard tracheostomy usually requires general anesthesia and carries a high risk of ventilator dependence in ALS patients suffering from respiratory failure. In contrast, PDT with a small uncuffed cannula is less invasive and may contribute to maintain the general welfare of the patient. PDT is regarded as beneficial to ensure a peaceful end and increase the quality of life of ALS patients and their families.

P142 A POPULATION BASED PILOT STUDY OF RESPIRATORY FUNCTION IN MOTOR NEURON DISEASE PATIENTS IN TAYSIDE AND NORTH-EAST FIFE: DEVELOPMENT OF A GUIDELINE/ REFERRAL PROTOCOL

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Background: Respiratory failure is the most common form of death for a motor neuron disease patient, with respiratory insufficiency symptoms often insidious in onset, implying a larger problem than is currently realized. There is evidence that symptomatic management by the use of non-invasive ventilation can improve a patient's quality of life; however, this form of treatment is not uniformly practised in the United Kingdom and the use of non-invasive ventilation falls behind that of the United States and Europe with this patient group. To our knowledge there have been no population-based studies to ascertain the level of the problem within a motor neuron disease population.

Objectives: To evaluate the level of respiratory insufficiency within the motor neuron disease population of Tayside and north-east Fife and to develop a protocol/

guideline for referral for symptomatic management of respiratory insufficiency.

Methods: A population based study carried out over a three-month period on all patients with a diagnosis of motor neuron disease as classified by the El Escorial criteria within the Tayside and north-east Fife area. Outcome measures used include spirometry, sniff nasal inspiratory pressure, nocturnal pulse oximetry measurements, venous bicarbonate and chloride levels, the Epworth Sleepiness questionnaire and the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale.

Results: Thirty-one patients were included in the study. Standard questionnaires such as Epworth Sleepiness Scale and Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised identified 26% and 16%, respectively, of cases with respiratory insufficiency symptoms. However, with the use of more detailed questions, the level of the problem increased with 87% of the study population reporting at least one respiratory insufficiency symptom. Fifteen cases (58%) had a spirometry result below 70% predicted value (four missing cases), and nocturnal pulse oximetry results showed that 11 cases spent $>5\%$ of time below 90% oxygen saturation. Using the proposed guideline/protocol 10 patients (32%) met the criteria for referral to the respiratory physician for further respiratory assessment and initiation of non-invasive ventilation if they chose this option; currently only one patient had been offered this symptomatic treatment.

Conclusions: The use of standard questionnaires is insufficient to detect respiratory compromise and more detailed questions were required in conjunction with respiratory function tests. The development of the guideline/protocol allows more effective monitoring of respiratory function with the possibility of use throughout Scotland. Through the use of the proposed guideline/protocol the quality of life for an estimated 84 people living with motor neuron disease in Scotland could be improved by referral for non-invasive ventilatory support.

P143 AMYOTROPHIC LATERAL SCLEROSIS: CORRELATION BETWEEN MAXIMUM PHONATION TIME AND FORCED VITAL CAPACITY

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Background: Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease in which progressive respiratory failure is usually the cause of death. Regular assessment of respiratory function is important in predicting disease progression. In particular, Forced Vital Capacity (FVC) is a useful prognostic index and its reduction below 50% of predicted value is often associated with the onset of respiratory symptoms.

Objectives and methods: In order to obtain a more simple, reliable and non-stressful measurement to evaluate the progression of ventilatory impairment even in patients at home, we investigated the relationship between maximum phonation time (MPT) and FVC. We considered data from 40 ALS patients (28 males, 12 females) followed at our Neuromuscular Unit in the period February 2004–February 2005. A motor involvement was present in all patients whereas bulbar symptoms were found only in 25 patients. At each quarterly examination, FVC was assessed by a portable spirometer and MPT was obtained by asking the patients to take a single deep breath and to prolong the vowel sound 'a' as long and steadily as possible. All subjects were tested while in seated position. For each examination the best of three attempts was considered for both FVC and MPT assessment.

Results: A regression analysis between MPT and FVC was performed and a strong positive correlation was found between the two variables in ALS patients whereas no significant correlation was found in the ALS patients with bulbar signs.

Conclusions: This result indicates MPT as an index of ventilatory function in ALS patients without bulbar involvement.

P144 EARLY RESULTS OF LAPAROSCOPIC MOTOR POINT DIAPHRAGM PACING IN AMYOTROPHIC LATERAL SCLEROSIS: CAN EXOGENOUS ELECTRICAL STIMULATION IMPACT RESPIRATORY FAILURE?

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Background: Respiratory failure is responsible for the majority of deaths in patients with amyotrophic lateral sclerosis (ALS). Therapeutic electrical stimulation has been shown to maintain the strength of other peripheral muscles in ALS. Electrical stimulation may also maintain physiologic activity, contractile properties, calcium levels and promote collateral axon sprouting. We have shown that a laparoscopic diaphragm pacing system in spinal cord injured patients is a low-risk, cost-effective outpatient system that will support the full-time respiratory needs of patients. We are currently studying the use of this technology in patients with ALS and respiratory insufficiency.

Objective: To evaluate, in this Phase I trial, the safety of implanting the diaphragm pacing system for use in conditioning the diaphragm of classic ALS patients. Our secondary objectives are to assess the system in slowing the respiratory decline and improve quality of life for implanted patients.

Methods: The principle inclusion requirements are a forced vital capacity above 50% at the start of the study and above 45% at the time of surgical implantation. Each patient is followed for three months pre-implantation with a series of tests including: pulmonary function tests, speech phonation times, ultrasound analysis of diaphragm thickness, phrenic nerve conduction tests and quality of life tests. Patients undergo a 1–2 h outpatient laparoscopic mapping of their diaphragm to locate the phrenic nerve motor points with two electrodes implanted in each hemidiaphragm. Two weeks after surgery, the stimulus output characteristics of each electrode are determined and stimulus parameters are selected for conditioning. The patients then condition their diaphragm at home with five 30-min sessions of therapeutic electrical stimulation per day. The conditioning sessions may be performed while they are carrying out their ordinary activities of daily living. Patients are assessed post-operatively on a monthly basis with the same tests performed pre-operatively to compare pre- and post-implant trends.

Results: Three patients were successfully implanted. All three had an FVC of 53% or less at the time of surgery. The diaphragms at surgery presented with significant weakness in two patients. One patient had a radial banding appearance of her intact motor units. All have been able to tolerate training with the system. At early follow-up the patients' FVC (without stimulation) has decreased but at the 2-month follow-up the first patient's FVC had increased to above the 1-month follow-up. In all patients the phonation times, muscle thickness and movement of diaphragm with stimulation under fluoroscopy have improved. All three patients have, anecdotally, reported feeling better.

Conclusion: The diaphragm pacing system can be safely implanted and utilized in patients with ALS. Whether there will be a long-term improvement in ventilation leading to an increased survival or quality of life requires further follow-up.

P145 RESPIRATORY AIDS IN PATIENTS WITH MOTOR NEURON DISEASE

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Background: Non-invasive ventilation (NIV), portable suction (PS), exsufflator/insufflator (EI) and high frequency chest wall oscillator (HFCWO) are respiratory aids (RAs) used in patients with motor neuron disease (MND).

Objectives: To determine use of RAs in our clinic, benefits of NIV and HFCWO, and patient features predicting use of NIV.

Methods: Retrospective study of MND patients seen over the past two years for whom the recommendation of a RA was made.

Results: Thirty-nine patients fit the criteria, and for all, NIV was the first RA recommended either alone or in conjunction with another RA. Thirty-two (82%) agreed to try NIV, and seven refused. Of those who refused initially, one agreed three months later, and one used it terminally as palliation. There was no significant difference in gender, onset site, sleep score, ALS-FRS-R, orthopnea or dyspnea score for those who agreed to NIV initially and those who did not. In 16 patients with sleep scores recorded pre- and post-NIV, it improved in 10, was unchanged in four, and worsened in two following NIV. There was no significant difference in the change in sleep score for patients who used NIV 0–4 h compared to those who used it longer. Of the 19 patients who were seen at least once after initiating NIV, at three months 12 (63%) were using NIV for 4–8 h per night. Of the seven using it less at three months, two ceased using it within a year and three subsequently increased use. Three months after starting NIV, patients who could identify specific improved symptoms were more likely to be using it 4–8 h per night ($p < 0.01$). Nineteen patients had HFCWO recommended for weak cough, three refused, one agreed but died before starting, and 15 tried it. For the 10 who used it for more than a month, using a 0–5 point scale with 0 'strongly disagree' and 5 'strongly agree', they reported at one month: improved secretion clearance 4.1 ± 0.6; less shortness of breath 3.9 ± 0.8, and better sleep 3.9 ± 0.7. NIV alone was used by 14, HFCWO and NIV, seven; suction alone, one; NIV and EI, three; NIV, suction, and HFCWO, three; NIV, suction, HFCWO, and EI three.

Conclusions: The percentage of our patients willing to try NIV and use it for more than 4 h per night was high. This is probably multifactorial, as two years ago a respiratory therapist joined our clinic, and we identified a home respiratory therapy company that provided excellent teaching and follow-up, and changed the brand of the NIV machine. We could not identify factors that distinguished between patients willing to try NIV and those unwilling; identifiable symptomatic benefits were associated with longer nightly use. HFCWO may be helpful to some patients with MND, particularly regarding secretion clearance.

P146 PREDICTING OUTCOMES FOR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS TREATED WITH NON-INVASIVE POSITIVE PRESSURE VENTILATION USING A SALIVARY DYSPHAGIA SCORE

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Background: Non-invasive positive pressure ventilation (NPPV) is often used to support breathing in patients with amyotrophic lateral sclerosis (ALS). However, patients with progressive bulbar paralysis may not tolerate NPPV due to their inability to swallow saliva. Thus, severe dysphagia threatens survival.

Objective: The purpose of this study was to develop a salivary dysphagia scoring system and evaluate the score's ability to 1) predict duration of survival after initiation of NPPV, 2) determine intolerance of NPPV, and 3) identify appropriate care interventions, including the need for end-of-life care, or when to start invasive ventilation.

Methods: Data were collected from 1990 to 2002 on 157 patients. Twenty-two tried NPPV, but failed. The remaining 135 were treated with NPPV and closely followed through ongoing periodic home visits. An ordinal scoring system was defined as follows: score of 4=no impaired ability to swallow saliva; 3=small accumulations of saliva in the mouth; 2=occasional pooling/drooling; 1=frequent pooling/drooling and conscious effort to swallow; 0=constant pooling/drooling plus the inability to clear secretions from the trachea. Survival was defined as the interval (months) from the start of NPPV until death or transition to TPPV. Intolerance of NPPV was defined as the inability to continue treatment without risk of aspiration. Median survival scores were compared with using Kruskal-Wallis one-way ANOVA. Post hoc comparisons were performed with Dunn's method. Differences associated with p values <0.05 were considered significant.

Results: All patients receiving NPPV had initial scores greater than zero. Median survival differed significantly between those with a score of 1 (eight months) compared to those with a score of 4 (27 months, $p=0.002$). Fifteen patients with a score of 4 used NPPV for 24 h per day and survived 24 to 92 months. Seven of the 15 used NPPV for 5 to 7+ years. There was no difference in survival between patients with scores of 2 and 3. The median score for patients who became intolerant of NPPV and died was 1. The use of medications to dry secretions or mechanically assisted coughing was ineffective in patients with a score of 0 or 1.

Conclusions: The salivary dysphagia score was useful in predicting outcomes on NPPV. A score of 1 reliably signaled the intolerance of NPPV and/or the need for end-of-life care or transition to invasive ventilation. The score is easy to apply and may help to avoid inappropriate therapy, emergency hospitalizations or early mortality.

P147 SURVIVAL AND FACTORS PREDICTING SURVIVAL FOLLOWING NON-INVASIVE VENTILATION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is characterized by a progressive decline of motor functions with involvement of respiratory muscles as a major predicting factor for survival. There is considerable evidence that non-invasive ventilation (NIV) can treat and relieve symptoms of alveolar hypoventilation.

Objectives: To determine survival and factors predicting survival following NIV in 33 patients with ALS.

Methods: We conducted a retrospective study of 33 ALS patients with NIV, followed between 1996 and 2004. We determined mean survival since onset of the disease and since treatment by NIV. Statistical analysis was performed using the Kaplan-Meier test. Cox proportional hazards models were constructed to adjust for potential confounding variables.

Results: Mean age was 60.4 years with 23 male and 10 female patients. Ten patients had bulbar onset, 22 patients had limb onset and one patient had initial (inaugural) respiratory signs. Mean initial and maximal diurnal total utilization of NIV were 10 and 14 h. Mean survival since ALS onset and since treatment by NIV were 34.2 and 8.4 months. Survival depends on age and clinical form. Survival after treatment by NIV is influenced by the use of a mechanical in-exsufflation device.

Discussion: Prognosis depends on age and initial clinical form of the disease. Survival after treatment by NIV is not influenced by respiratory parameters or bulbar symptoms. The utilization of thermoformed nasal interfaces is accompanied by an excellent tolerance of NIV, independent of the clinical form.

Conclusion: NIV can treat sleep-disordered breathing and nocturnal alveolar hypoventilation, also in bulbar onset ALS patients. Clarification for optimal timing for initiation of NIV and tolerance needs to be undertaken.

P148 THE RESPIRATORY SUPPORT OF ALS PATIENTS WITH TPPV IN TOKYO METROPOLITAN NEUROLOGICAL HOSPITAL FROM 1980 TO 2005

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Background: The clinical investigations of ALS patients using tracheostomy positive pressure ventilation (TPPV) in Tokyo Metropolitan Neurological Hospital (TMNH) have disclosed the total manifestation of ALS. In this study, we re-examined various problems of ALS patients with TPPV in TMNH from 1980 to 2005.

Objectives and methods: To investigate the clinical course of ALS with TPPV, we examined the medical records of ALS patients admitted to TMNH from January 1980 to May 2005. Kaplan-Meier analyses were used to estimate the disease prognosis.

Results: Of the 239 ALS patients who showed critical respiratory failure between 1980 and 2005, 98 patients (61 males and 37 females) were treated with TPPV (41%), while 141 patients died without ventilation support. In the early years (from 1980 to 1984), however, almost 100% of patients were treated with TPPV. The median interval from onset to respiratory failure was 28.6 months in the 98 patients with TPPV. Sixty-one patients of the 98 patients have already died, and the median survival period of them was 128.6 months. In the 41 patients on TPPV for more than five years, severely impaired communication occurred, namely the totally locked-in state (TLS) developed in seven (17.1%) and the minimal communication state (MCS) in 12 (29.3%). The causes of death were pneumonia (23 patients, 38%), heart failure (eight patients, 13%), sudden cardiac arrest (seven patients, 11%), renal failure (three patients, 5%), and others. Although 36 patients among 61 deceased patients were followed up at home by the neurologists from our hospital, only two patients died at home and others died in our hospital or other hospitals.

Discussion and conclusions: The high frequency of TPPV treatment in the first five years and gradual decrease to plateau level (around 40%) in TMNH might be partly attributed to a lack of provision of precise information to the patients in the early period, but afterwards we tried to disclose to the patients the diagnosis, and the information about the likely course of ALS based on the 'the new view of ALS'. Although the ALS patients with TPPV and caregivers need to cope with various burdens due to aggravating motor impairments after respiratory failure, it has been confirmed that they could live for a long time beyond respiratory failure. About 50% of patients on TPPV showed severely impaired communication (MCS or TLS); however, many patients led active lives, expressing their thoughts using communication devices for many years. To improve prognosis further, we have to prevent pneumonia effectively and pay attention to the sympathetic hyperactivity which may cause circulatory collapse and sudden death. The low frequency of death at home in spite of the high percentage of follow-up by neurologists at home may reflect Japanese socio-cultural circumstances.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 146–151

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THEME 8 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

P149 EMOTIONAL CONFLICTS OF PATIENTS WITH AMYTROPHIC LATERAL SCLEROSIS ON WEARING INVASIVE VENTILATORS

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Objectives: This study aimed to reveal emotional conflicts of patients with ALS before and after wearing invasive ventilators.

Methods: Semi-structural interviews were conducted with 20 subjects (11 patients and 9 family members) in three areas of Japan.

Results: The following results were obtained: 1) most patients felt shocked when they were told about their successive respiratory dysfunction, 2) patients wondered whether they should use ventilators or not, and it took them up to four years to make this decision, 3) five out of six ventilator users felt resistance to wearing ventilators, but four of them accepted use later with the support of their families, peers and health providers.

Conclusion: Emotional conflicts surrounding decision-making on the use of ventilators is one step on the path of coping with ALS. Adequate support from patients' families, peers, and health providers is important for a smooth decision-making process.

P150 PSYCHOLOGICAL AND PSYCHOPATHOLOGICAL APPROACH TO PATIENTS AFFECTED BY AMYOTROPHIC LATERAL SCLEROSIS

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Objectives: To examine the relationship between patients' personality traits and the evolution of their disease. Our hypothesis is that family relations form the basis for mood alterations and that these are associated with personality changes in patients suffering from ALS. Secondary aims sought to examine how the patient-caregiver relationship affects patients' average survival time, to study the prevalence of symptoms such as anxiety and depression in patients with ALS, and the defensive mechanisms commonly employed. Finally, we sought to evaluate how psychological support for both the patient and caregiver can improve their quality of life.

Methods: Twenty patients suffering from ALS and 20 caregivers were administered psychodiagnostic interviews with a focus on psychopathological symptoms, in addition to the Eysenck Personality Inventory (EPI) scale, State Trait Anxiety Inventory (STAI) scale and Hamilton Depression (HDS) scale.

The data were statistically analysed by two-tailed Student's *t*-test (independent samples design). Exclusion criteria for all subjects included dementia and alterations of cognitive function.

Results and conclusions: The psychological evaluation showed that patients suffering from ALS have higher scores on the STAI, as well as higher levels of somatic anxiety and depression. They present a rigid defensive system and their survival time is correlated with quality of life.

P151 HOPE AND ALS

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Background: The assumption that a diagnosis of ALS leads to hopelessness and depression is prevalent in society and among health care professionals. The absence of a cure conjures up images of suffering and death. Recent studies of symptom palliation and effective disease management have begun to challenge this perception. Nevertheless, questions remain about the appropriate point at which to reveal diagnoses and the seriousness of disease progression. Clinicians fear that truthfulness may take away a patient's hope, but we are unaware of studies that have specifically examined hopelessness or optimism in ALS.

Objectives: To examine hopelessness over the course of ALS from diagnosis to death.

Methods: Two cohorts were assessed: 1) patients who were assessed within one year of diagnosis (n=90), 2) late-stage patients with high likelihood of dying or need for tracheostomy within six months (n=80). Both completed

ISSN 1466-0822 print/ISSN 1471-180X online © 2005 Taylor & Francis
DOI: 10.1080/17434470510045069

10 items from the Beck Hopelessness Scale (BHS, range 0–10; 10=maximum hopelessness). Mean hopelessness scores were computed. Late-stage patients were interviewed monthly as they approached death or tracheostomy. Patients also completed the Beck Depression Inventory (BDI).

Results: Complete BHS data were available for 65 of the recently diagnosed cohort and 60 of the late-stage cohort. Patients completing and not completing the measure did not differ in sociodemographic or disease characteristics. Mean BHS scores for the two cohorts did not significantly differ (3.54 among recently diagnosed patients versus 3.58 among late-stage patients). These scores fall in the mild range. By contrast, BDI scores were significantly higher in the late-stage cohort (13.2 vs. 9.9; $p=0.006$), although mean scores in both groups fell below thresholds for clinical significance. In the three months prior to death or tracheostomy, hopelessness scores were mostly stable (3.8, 3.2, 3.8 in people who died; 3.3, 3.5, and 3.0 in people who chose tracheostomy ($n=7$)).

Discussion: Empirical findings challenge the assumption that hopelessness increases over the course of ALS. The absence of an increase of hopelessness with disease progression and the approach of death suggests that patients cope with increasing disability and new challenges in disease management. These findings confirm reports of the general resiliency of patients. Additional research is required on patient reactions to physician communication of threatening information and patient coping strategies.

P152 THE PREVALENCE OF DEPRESSION AND ANXIETY IN MND

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Background: There is an impression both in clinical practice and the research literature that patients with MND are typically ''cheerful, stoical, and positive''. Estimated prevalence rates of clinically significant anxiety and depression in MND are typically low, around 5–10%. This is in marked contrast to higher estimated rates in other neurodegenerative movement disorders such as multiple sclerosis and Parkinson's disease where estimates are around 40–60%. It is not clear whether reliance on specific interview methods may have contributed to differing estimates of affective disorder in people with MND as studies have used varying assessment tools.

Objectives: To estimate prevalence rates of depression and anxiety in MND using different assessment tools.

Method: Three self-report questionnaires, the Beck Depression Inventory (BDI), Hospital Anxiety and Depression Scale (HADS), and the Spielberger State-Trait Anxiety Inventory (STAI) were sent to a 12- month consecutive sample of non-demented MND patients attending a tertiary referral clinic in the UK. Standard cut-off scores were used to identify caseness.

Results: From 190 questionnaire packs sent out, 101 have been returned completed, yielding a 53% response rate. BDI scores reveal that 46% were classified as not depressed, 36% were mild-moderately depressed, 13% were moderately-severely depressed, and 5% were severely depressed. The HADS Depression subscale identified that 75% were not depressed, 13% were borderline, and 12% were depressed. STAI State Anxiety identified that 58% had low anxiety levels, 33% had medium anxiety levels, and 8% had high anxiety levels. The HADS Anxiety scale revealed that 65% were not anxious, 17% were borderline, and 17% were anxious. Depression and anxiety scores were both moderately correlated with levels of pain experienced by patients. Despite the presence of psychological distress, 74% of patients were not taking any antidepressant medication, and those patients who were using antidepressant medication had significantly higher levels of both depression and anxiety.

Conclusion: The prevalence of affective disorder amongst MND patients may be higher than previously estimated. However, prevalence estimates for depression vary according to the measure employed, with the BDI producing higher estimates than the HADS. It should also be noted that self-report questionnaires provide an estimate of symptom frequency, rather than a clinical diagnosis. Nevertheless, their utility in clinical practice should be considered and future research should examine the efficacy of antidepressants and/or psychosocial interventions in MND.

P153 COGNITIVE LINGUISTIC DEFICITS, QUALITY OF LIFE AND DEPRESSION IN ALS PATIENTS: A CROSS-SECTIONAL STUDY

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Background: Cognitive symptoms in ALS often go undetected because they are elusive and may be overshadowed by the more apparent physical symptoms. Nevertheless, cognitive deficits are present in ALS although often underestimated. In particular, cognitive linguistic symptoms may be disguised by speech impairment caused by bulbar symptoms. Cognitive linguistic deficits can undermine patients' quality of life, particularly if they go undetected by family members and health professionals. In addition, patients' ability to understand and communicate is essential when crucial decisions about their management are to be taken during the course of the disease.

Objectives: To detect cognitive linguistic deficits in ALS patients and identify any correlations with quality of life and depression.

Methods: Forty-five patients were administered part of an Italian battery, the BADA (Batteria Analisi Deficit Afasici, 'Battery for the Analysis of Aphasic Deficits'), comprising four domains: reading comprehension, detection of errors in a text read aloud by the interviewer, number of grammar and spelling mistakes made writing a text, and verbal description of some pictures. Each patient was given the part of the battery they were able to do, depending on whether their writing or speech abilities were preserved. In addition, they were administered the Zung Depression Scale (ZDS) and the McGill Quality of Life Questionnaire (MQoL). Patients' physical status was assessed by the ALS Functional Rating Scale (ALS-FRS). Pearson bivariate correlations were carried out using Statistical Package for Social Science (SPSS) version 12.0 for Windows. A significance level of $p<0.05$ was used.

Results: Forty-five patients were recruited, 31 (69%) of whom were males, and 14 females (31%); 16 (36%) had a bulbar onset, and 29 (65%) a spinal onset. Mean age was 63.0 years (SD 9.8). Mean ALS-FRS score was 28.8 (SD 7.1). Mean ZDS score was 42.4 (SD 9.5), with 10 subjects over the 50-point cut-off score indicating overt depression. Mean MQoL score was 6.9 (SD 1.3). Significant correlations were found between: the detection of grammar mistakes in a text read aloud by the interviewer and the ZDS ($p<0.01$); the frequency of grammar mistakes and the site of onset ($p<0.05$); and existential well-being as measured by MQoL and frequency of spelling mistakes ($p<0.05$). All cognitive linguistic deficits correlated with age and education, except spelling mistakes, which correlated only with age.

Conclusion: The presence of cognitive symptoms in ALS is somewhat underestimated, partly because they do not stand out as the patient's main problem, and partly because they are difficult to detect, particularly in bulbar patients. Moreover, early symptoms of cognitive impairment might be mistaken for depression, which would not be surprising in someone affected by such a disease. Discriminating between depression and early cognitive impairment may be useful in enhancing patients' quality of life.

P154 FORCED CHOICE COGNITIVE BATTERY IN PATIENTS WITH MOTOR NEURON DISEASE

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Background: In recent years, cognitive changes have been reported with increasing frequency in motor neuron disease (MND). Physical changes related to MND may impact test performance and limit the usefulness of timed and written tests. Lengthy neuropsychological testing may prove challenging for a patient who already spends several hours in the multidisciplinary clinic.

Objective: To perform a pilot study of our cognitive battery (CB) in patients with MND in order to determine if it could be administered in less than an hour, and to patients in all stages of disease. We selected brief, untimed, forced choice tests, allowing for eye blink responses if necessary. Some tests had to be modified to fit these criteria. We were also interested in whether performance correlated with disease duration, depression, functional status as measured by ALS-FRS-R or forced vital capacity (FVC).

Methods: Patients performed the following tests at baseline, six months and 12 months: Matrix Reasoning (MR), Peabody Picture Vocabulary Test (PPVT), anti-saccades (AS), modified CERAD word list recognition (mC), visual analogue scale for depression (VAS-D), and modified orientation (mO). The neuropsychiatric inventory (NPI) was administered to caregivers. Patients had FVC tested and ALS-FRS-R carried out at each visit.

Results: Thirteen patients completed the baseline CB; of these, six have also carried out the six-month assessment, and one has completed the full 12-month study. One patient failed to do their six-month study because of medication-related sleepiness; all other patients could perform all parts of the CB. Duration of disease at time of the baseline exam was 45.9 ± 44.8 months (range 8–156). Baseline results were as follows: AS (out of a possible 10) 8.6 ± 1.7; scaled MR 11.2 ± 3.2; standardized PPVT 104.1 ± 10.7; mC 9.0 ± 1.7; mO (out of a possible 6) 6 ± 0. The number of patients who scored at least one standard deviation below the normal mean at baseline included two patients on PPVT, four patients on mC, and five patients on AS. On the NPI, caregivers reported abnormalities in four patients, and three of these had at least one other abnormal cognitive test. Three patients had an entirely normal CB. ALS-FRS-R scores ranged from 10 to 45, with a mean score of 34.3 ± 9.8. Time to complete the test (exclusive of the NPI) was 35 ± 8 min (range 25–50 min). Using a simple linear regression model, there was a relationship between FVC and MR, FVC and percentile score on PPVT, and ALS-FRS-R and MR. There was no relationship between depression or disease duration and CB results.

Conclusion: Our CB could be administered to all patients, regardless of the severity of their disease, in less than an hour. Neither disease duration nor depression played a role in cognitive test results, while respiratory and overall functional status may be a significant influence.

P155 RESEARCH IN AMYOTROPHIC LATERAL SCLEROSIS: INSIGHT OF COGNITIVE FEATURES AND AFFECTIVITY

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Background: Amyotrophic lateral sclerosis (ALS) has not previously been assessed with a 'projective' exam such as the Rorschach Inkblot test.

Method: The Rorschach protocols of 20 patients affected by ALS were examined, using Exner's system of scoring indices. This study evaluated both affective variables of patients and their cognitive information processing, comparing their results to data from the Exner Comprehensive System normative sample; statistical analysis was performed with Student's *t*-test.

Results: Patients' responses revealed a significantly abnormal form of perception in the screening of reality, as well as an increased prevalence of disturbed thinking (revealed by high number of 'special' scores), inaccurate perceived human responses (M-) and a higher frequency of reservations. These results suggest impairment of attentional system and cognitive deterioration. Moreover, some of these aspects are linked to psychotic symptoms. Responses also revealed an increased percentage of pure forms (F%) and low affective ratio, which reflects the avoidance of emotional interchange with environment, lack of intense feelings, rigidity on own opinions, ruminative self-introspections and shame, depressive mood, low self-esteem, no interest for other people, lack of expectations of close relationship with others, negation of own primary needs, self-concept thought as partial, and high attention in physical conditions.

Conclusion: Affective features of Rorschach test demonstrate that ALS patients feel a progressive deterioration and are adapting to death, retiring from the outside world. Cognitive aspects are also of interest; Exner Comprehensive System could be used to detect early frontal dementia.

P156 FRONTOTEMPORAL DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS: A REVIEW OF CURRENT CONCEPTS

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Background: The presence of dementia in a proportion of ALS patients is increasingly being recognized. Clinical, functional imaging, genetic and pathological studies indicate a continuum of cognitive and behavioural changes among ALS patients that can progress to dementia in some.

Objectives: The present study examined the prevalence of cognitive and behavioural impairment in a sample of ALS patients. Findings will be presented and the current research on FTD and ALS will be reviewed.

Methods: Twenty males and females diagnosed with definite ALS were recruited from the Kessenich Family MDA ALS Center in the Department of Neurology at the Miller School of Medicine, University of Miami. Participants were administered a neuropsychological test battery and a caregiver was asked to rate the patient on a behavioural survey.

Results: Approximately half of the participants were cognitively intact and exhibited no behavioural changes. Two patients were diagnosed with FTD according to the Neary criteria (1). The remaining patients had varying degrees of cognitive and behavioural changes consistent with subclinical levels of FTD. Results of Pearson correlations indicate that degree of cognitive impairment was significantly correlated with severity of bulbar involvement measured by the ALS Functional Rating Scale ($p < 0.05$).

Discussion: The prevalence of cognitive and behavioural changes in this sample of ALS patients is consistent with previous reports. Our hypothesis is that cognitive changes correlate with the severity of bulbar involvement irrespective of whether bulbar involvement started at the outset or only developed later in the course of the disease. First, we will review methodology and results from the current and previous neuropsychological studies on ALS and FTLD, including correlations with pathological data; secondly, longitudinal neuropsychological studies in ALS and the methodological challenges involved in conducting such studies; and finally, the results of our current work on patients' willingness to accept percutaneous endoscopic gastrostomy (PEG), and non-invasive positive pressure ventilation (NIPPV) (2). The impact of cognitive impairment on patients' underutilization of PEG and NIPPV (3) will also be discussed.

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P157 THE ASSOCIATION OF FRONTOTEMPORAL DYSFUNCTION AND REDUCED VITAL CAPACITY IN ALS

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Background: ALS is recognized as a multi-system disorder and impairments of cognitive function have been consistently demonstrated. While the cognitive dysfunction may be related to breathing problems associated with sleep disturbances, the effect of hypoventilation on the cognitive impairments in ALS patients is still unclear.

Objective: To evaluate the association of frontotemporal dysfunction and reduced vital capacity in ALS.

Method: We measured disability with the ALS Functional Rating Scale-Revised (ALSFRS-R), Forced Vital Capacity (FVC) and cognitive functions including attention, executive function, and memory in 16 ALS patients (10 males, six females, aged 43–75 years, mean 57.6 $\pm$ 10.5 years, ALSFRS-R 27–44).

Results: The patients were grouped based on FVC. The first group (n=8, mean ALSFRS-R=37) showed reduced FVC ($\leqslant$80% of predicted value) and the other group (n=8, mean ALSFRS-R=37) had FVC within normal limits. The groups did not differ significantly in sex, age, education and ALSFRS-R. The first group performed significantly worse in memory retention (p=0.028), retrieval efficacy (p=0.003), spoken verbal fluency (p=0.03) and spoken verbal fluency index (p=0.016) than the second.

Conclusion: Reduced vital capacity is correlated with poor performance in tests reflecting fronto-temporal functions. Therefore, the frontal and/or temporal impairments in ALS might be attributable to potentially reversible secondary effects of hypoventilation, as well as the primary degenerative process observed in the frontotemporal dementia-ALS complex.

P158 PLANNING DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Background: Executive functions allow coping with new and complex situations. The Tower of London (TOL) was initially developed by Shallice (1) to predict planning problems arising from the dysexecutive syndrome. There is great variability in the extent and degree of impairment in patients with frontal lobe damage. Executive dysfunction has been described in patients with amyotrophic lateral sclerosis (ALS), with frontotemporal dementia (FTD) and in patients with ALS without dementia. However, no study has specifically described the planning function. The study of the link between ALS and FTD appears to be clinically relevant.

Objectives: To characterize the planning function in patients with ALS with and without cognitive deficits (mild cognitive deficit or FTD) and to determine if the TOL (modified version) can discriminate between these two categories of patients.

Methods: We studied the planning performance of 11 ALS patients with FTD (ALS/FTD), 10 patients with cognitive impairment (but not demented) (ALS/CI) and 12 ALS patients without cognitive impairment, compared to 27 age-matched controls. A modified manual version of the TOL with three different height disks on three rods of different lengths was used in this study. The object is to change the arrangement of the disks to match an arrangement predetermined by the experimenter in the fewest possible moves while respecting certain rules. The subject was given 12 problems with four levels of difficulty (2-, 3-, 4-, and 5-move levels of difficulty). Five measures were considered: the percentage of perfectly resolved problems (in the maximum allowed moves), planning time, movement (or solution) time, the mean number of moves above the minimum and the number of rule breaks.

Results: Except for the planning time, ALS/FTD and ALS/CI patients generally performed more poorly than both ALS patients and controls as the task difficulty increased. Their respective difficulties were quantitatively and qualitatively distinct. According to the levels of difficulty, ALS/FTD patients could resolve fewer problems, spent more time on problems and made more moves than the ALS/CI patients, ALS patients and controls. Apart from in the easier 2-move level of difficulty problems, ALS/FTD patients broke the rules more often than ALS/CI, ALS and controls for all the levels of difficulty. ALS/CI patients' difficulties appeared less marked than those of ALS/FTD patients. They performed more poorly than controls but not significantly worse than ALS patients without cognitive impairment. ALS patients without cognitive impairment resolved only the 3-move level of difficulty problems slower than control participants. Motor deficits could not be considered as biasing performance.

Conclusion: The TOL task was described to be useful for the assessment of problem-solving and provided a tool for picking up difficulties in planning. Its use appears to be interesting in the ALS population, in which executive dysfunction is increasingly recognized

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P159 PREMORBID PERSONALITY CHARACTERISTICS OF PATIENTS WITH ALS: PRELIMINARY FINDINGS

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Background: Physicians experienced in treating patients with ALS have often described these individuals as being especially ''nice'', noting their remarkable resilience in coping with this relentlessly progressive disease. Previous research studies on the personality characteristics of ALS patients have yielded mixed results.

Objective: The purpose of the present study was to further investigate whether a characteristic premorbid personality profile is associated with ALS.

Methods: Forty-nine caregivers of newly diagnosed (<six months) ALS patients were recruited for study participation from the Kessenich Family MDA ALS Center in the Department of Neurology at the Miller School of Medicine of the University of Miami. The control group ($n=47$) consisted of caregivers of patients newly diagnosed with lung cancer ($n=22$), multiple sclerosis [MS] ($n=15$), and brain glioma ($n=10$). Caregivers completed the NEO-Personality Inventory and were asked to rate the patient's personality characteristics as they were before receiving their current medical diagnoses.

Results: Results of a multivariate analysis of variance indicated that ALS patients were rated as being significantly lower in the Openness personality trait ($F=3.99$, $p<0.01$) than controls. A univariate analysis of variance was conducted to further examine group differences in Openness. There was a statistically significant difference between the groups ($F=3.37$, $p<0.05$), with the ALS group having the lowest mean.

Conclusion: Preliminary findings indicate that there are differences in premorbid personality characteristics between patients with ALS and those with other chronic diseases. In our study, caregivers rated patients with ALS as being lower in the Openness personality trait than did caregivers of patients with other chronic medical conditions. Individuals who are low in Openness attend to the task at hand, exert control over their emotions, prefer familiarity and routine, and hold traditional values. This personality feature appears to serve an adaptive function in terms of coping with ALS, and to physicians it may cause the patient to appear pleasant, cooperative, and resilient. The exact relationship between the genetic basis of these personality traits and the genes predisposing to or protecting against the development of ALS is uncertain at this time. Future research studies will be needed to examine the association between premorbid personality and the development of aspects of FTD as they impinge on the management and health outcomes in ALS.

Amyotrophic Lateral Sclerosis. 2005 (Suppl 1); 6: 152–165

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THEME 9 RESEARCH TO IMPROVE STANDARDS OF CARE

P160 NEUROPROTECTIVE AGENTS FOR CLINICAL TRIALS IN ALS: A SYSTEMATIC ASSESSMENT

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Background: Therapies that slow the progression of ALS are a crucial unmet need. Riluzole is currently the only FDA approved treatment for ALS, but its effect on survival is modest.

Objectives: The authors sought to identify potential neuroprotective agents for testing in phase III clinical trials and to identify which data need to be collected for each drug.

Methods: One hundred and twelve compounds were identified by inviting input from academic clinicians and researchers and via literature review to identify agents that have been tested in ALS animal models and in human ALS patients. The list was initially narrowed to 24 agents based on an evaluation of scientific rationale, toxicity and efficacy in previous animal and human studies. These 24 drugs then underwent more detailed pharmacological evaluation.

Results: Twenty drugs were finally selected as suitable for further development as treatments for ALS patients. Talampanel and tamoxifen have completed early phase II trials and have demonstrated preliminary efficacy. Other agents (ceftriaxone, minocycline, ONO- 2506 and IGF-1 polypeptide) are already in phase III trials. Remaining agents (AEOL 10150, arimoclomol, celastrol, coenzyme Q10, copaxone, IGF-1 – viral delivery, memantine, NAALADase inhibitors, nimesulide, scriptaid, sodium phenylbutyrate, thalidomide, trehalose) require additional pre-clinical animal data, human toxicity and pharmacokinetic data concerning CNS penetration prior to proceeding to large scale human testing. Further development of riluzole analogues should be considered.

Conclusions: Several potential neuroprotective compounds, representing a wide range of mechanisms, are available and merit further investigation in ALS.

P161 COMPLICATIONS IN QUANTITATIVE MUSCULAR ASSESSMENT (QMA) FOR ALS PATIENTS

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Background: In the course of ALS, the capacity of muscle to exert force is the most direct measurement of the natural history of the disease. For this reason, muscle strength is the outcome measure that provides the most useful information in ALS clinical trials. Our previous studies concluded that outcome measures in ALS clinical trials should include QMA because the Medical Research Council Scale is insensitive to small changes.

Objectives: The purpose of the study was analyse possible complications in quantitative muscular assessment (QMA) in ALS patients.

Methods: Observational Retrospective Study. The inclusion criterion was a diagnosis of probable or definitive ALS (El Escorial diagnostic criteria). QMA for ALS patients attending Hospital Carlos III (Madrid, Spain) over two years were evaluated. The QMA protocol included measurement of shoulder abduction-adduction (supine decubitus); elbow flexion-extension (supine decubitus); knee flexion-extension (sedestation) and grip strength. Tests were performed in both limbs using an electronic strain gauge tensiometer and a electronic Jamar dynamometer. Statistical analysis was conducted, establishing the absolute and relative proportions, with SPSS 10.0 for Windows.

Results: One hundred and thirty-nine tests have been analysed for a total of 51 patients during the period of study. The main complications that patients presented were: fatigue post-test (86%), episode of transitory cerebral ischaemia of haemorrhagic character (0.7%; $n=1$), transitory dyspnoea (12%). Tests had to be interrupted in 5% of cases due to impossibility to maintain the supine decubitus.

ISSN 1466-0822 print/ISSN 1471-180X online
DOI: 10.1080/17434470510045078

Conclusions: QMA are sure tests. Only in a minimum percentage of cases are the complications serious (0.7%).The most frequent complications are fatigue post-test (86%) and transitory dyspnoea (12%). Design of an informed consent for the QMA is necessary.

P162 DESIGN OF AN INFORMED CONSENT DOCUMENT FOR QUANTITATIVE MUSCLE ASSESSMENT (QMA) IN PATIENTS WITH ALS

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Background: In the last 30 years, the clinical relationship between health professionals and patients has been rather modified. There are several factors that have contributed to this change: 1) Development of new techniques; 2) Recognition of patients' autonomy to decide about their own lives, health and their own bodies; 3) The increasing number of lawsuits, complaints and judgments about the problems that clinical information involves, particularly the informed consent in clinical practice.

Patients should be informed and make decisions together with the health professional on each health care assessment procedure that is carried out. An informed consent document is necessary for QMA since the tests require the patient's maximum effort and therefore they bear a series of risks or complications that the patient should know and accept.

Objectives: To design an appropriate informed consent document (ICD) for the realization of the QMA in patients with ALS.

Methods: In the elaboration of the ICD a series of stages were followed: 1) Initial design of the document by a group of health professionals ($n=5$); 2) Revision of the document for a pedagogue; 3) Focal group of patients ($n=10$); the document was explained and patient understanding was assessed; 4) The document was revised carrying out the necessary changes.

Results: The final text includes the following aspects: patient's personal data; name of the physiotherapist who informs; the name of the procedure that will be carried out, with simple explanation of the objective of the procedure, what it consists of and the form in which it will be carried out; a description of the known consequences of the intervention, whenever they are considered excellent or important.

There is also a description of the typical risks of the procedure and a description of the personalized risks: relating to the personal circumstances of the patient with reference to their previous state of health, age, profession, beliefs, values and attitudes, or other circumstance of a similar nature. Information can be included that makes reference to the probable nuisances of the procedure and its consequences. It is made clear that the physiotherapist will expand on the information at the patient's request.

The patient declares that they have received information about the procedure, as indicated in the previous sections, as well as of alternatives to the procedure (manual muscle assessment) and acknowledges satisfaction with the received information and of having obtained explanation on their outlined doubts, as well as of their right to withdraw the ICD at any time without expressing a reason.

A section is provided for the date and the physiotherapist's and patient's signatures. A section for the consent of a legal representative, in the event of the patient's inability is included, as is a section for the repeal of the consent.

Conclusions: The final ICD for the realization of the QMA is based on consent among professionals and it has been revised by the patients, improving its understanding.

P163 THE PREVALENCE AND MANAGEMENT OF BURNING MOUTH SYNDROME IN MOTOR NEURON DISEASE

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Background: Burning mouth syndrome (BMS) has been described as burning sensations in the oral cavity, tongue or oral mucous membranes in the absence of clinical abnormalities (1). The most common areas in the mouth that are affected are the anterior two-thirds of the tongue, the lips and the anterior hard palate (2).

Objective: The aim of the study was to explore the association of burning mouth syndrome with motor neuron disease looking for causative factors and to determine treatment options.

Methods: MND patients attending the multidisciplinary clinic were asked about the existence of symptoms that may suggest burning mouth syndrome.

Results: Fourteen percent of patients reported symptoms consistent with BMS, significantly higher than the 1–5% previously reported for the general population. In these patients nutritional deficiencies such as vitamins B1, B2, B6 and zinc, and candidal infections were excluded. There were no clinical features or investigation findings to suggest Sjogrens syndrome, nor had any of the MND patients received radiotherapy. Of these patients with burning mouth symptoms, 27% had bulbar presentation. In total 73% developed bulbar involvement during the course of their MND. A trial of amitriptyline (10 mg)

was commenced in 40% of patients due to the severity of their presentation, with improvement in their symptoms.

Conclusion: BMS occurs in a significant proportion of MND patients. Symptoms of dysaesthesia and pain may reflect ectopic activity in denervated bulbar muscles. When severe, a trial of low-dose amitriptyline (10 mg) may be beneficial.

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P164 OPEN-LABEL COMPARATIVE STUDY OF AMITRIPTYLINE AND DYSPORT EFFICACY IN CONTROL OF SIALORRHOEA IN ALS

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Background: Amitriptyline for sialorrhoea is one of the 'gold standards' in motor neuron disease (MND), but its high doses worsen quality of life due to side-effects, requiring a reduction in dose and additional approaches to treatment.

Objectives: We conducted an open-label comparative trial of amitriptyline and dysport efficacy in MND patients with sialorrhoea.

Materials and methods: We studied 10 patients with MND with bulbar involvement (eight females and two males, age range 46–69 years) diagnosed by El Escorial criteria (1998), electromyography and MRI. Five patients took 25–100 mg/d of amitriptyline (group 1), and five patients received subcutaneous dysport injections (group 2) in 250 U total dose within projection of parotids and one submandibular gland. Before and two weeks after treatment all patients underwent gravimetry of saliva (cotton pillows weighted on electronic scales Sartorius CP225D, Germany, precision 0.01 mg). Side-effects of amitriptyline were assessed by questioning whether patients had experienced at least two of the symptoms.

Results: Sialorrhoea assessed by gravimetry either after amitriptyline (1.43 ± 0.39 and 0.91 ± 0.61 ml\5 min; $p < 0.05$), or dysport (1.11 ± 0.56 and 0.82 ± 0.42 ml\5 min; $p < 0.05$) was significantly lower than before treatment. Three patients developed more then two amitriptyline side-effects. The dose was lowered (25 mg), then dysport was injected. Gravimetry two weeks later showed that sialorrhoea remained decreased (0.56 ± 0.25 и 0.49 ± 0.29 ml\5 min; $p > 0.05$).

Conclusions: In cases of sialorrhoea in MND that require doses of amitriptyline causing side-effects, the lowering of amitriptyline dose and additional dysport therapy is indicated.

P165 GASTROSTOMY IN MND: AN AUDIT OF PRE-OPERATIVE ASSESSMENT

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Background: Evidence-based guidelines for the management of motor neuron disease were published by the American Association of Neurologists in 1999 (1). Based on evidence from retrospective cohort studies, the following guidelines were issued: 1) PEG is indicated for patients with ALS who have symptomatic dysphagia and should be considered soon after symptom onset; 2) For optimal safety and efficacy, PEG should be offered and placed when the patient's forced vital capacity (FVC) is more than 50% of predicted.

Objectives: To examine local practice with respect to gastrostomy in MND: to determine whether FVC is being measured pre-operatively, and to look at mortality post-gastrostomy.

Methods: Audit of patient records, 1/2000 to 1/2005, for patients with MND admitted for gastrostomy to a large district general hospital with neurology services. Data were available for 22 subjects.

Results: Seventeen out of 22 patients underwent PEG tube placement, 5/22 RIG. Thirty-day mortality was 23%. FVC was recorded prior to gastrostomy in 13% of patients.

Discussion: Mortality rates are similar to published rates from the Scottish Register of Motor Neurone Disease (25% 30-day mortality (2)). Forced vital capacity is not being recorded in most cases. Clearly clinicians are not adhering to the AAN guidelines. This may reflect a reluctance of clinicians to adopt guidelines issued without a firm evidence base. However, it may reflect poor awareness of the guidelines and a lack of understanding of the role of spirometry both in determining prognosis in MND, and in informing management decisions surrounding PEG, RIG or NG tube feeding.

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P166 DIAGNOSTIC DELAY IN MOTOR NEURON DISEASE: AN AUDIT

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Background: Motor neurone disease (MND) is a rapidly progressive and invariably fatal disease. Although early diagnosis is imperative, current evidence reports an average 11–22 month delay from symptom onset to diagnosis (1).

Objectives: In line with existing research, the MND service aims to diagnose 95% of patients within 12 months of symptom onset. This audit investigated the above standard by looking at the mean length of time taken for patients to receive a proposed and actual diagnosis of MND as well as the percentage of patients gaining a diagnosis within 12 months of symptom onset.

Method: We took the last 80 referrals to the MND service. Retrospective data were collected from hospital records.

Results: Only those patients diagnosed with MND at the regional centre were selected for inclusion in the audit (n=45), other cases were discarded; 68.2% had a diagnosis of MND proposed within 12 months of symptom onset and 60% had a diagnosis of MND confirmed within 12 months of symptom onset. This was below the set target of 95%. The mean time for confirmation of diagnosis was 13.3 months, which was well below the national average (1).

Conclusions: This audit found that while the majority of patients received a diagnosis of MND within 12 months, there were still a number of patients who did not receive their diagnosis within this time limit. There was often a considerable time delay between a proposed diagnosis being confirmed as definite MND. The wait for diagnostic neurophysiological studies and vague presenting symptoms were the most easily identifiable factors contributing to this diagnostic delay.

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P167 TIMING OF PEG, RESPIRATORY CARE, AIDS AND ADAPTATIONS: HOW ARE PEOPLE WITH MND/ALS SUPPORTED BY THE REHABILITATION TEAMS?

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Background: In the Netherlands specialized ALS care teams are merged into a national network. A consensus definition of a specialized ALS care team containing eight criteria was achieved in an expert meeting. Patients with ALS receive rehabilitation treatment according to the guideline 'the rehabilitation treatment of people with ALS'. In this guideline only general recommendations regarding the timing of PEG, respiratory care and aids and adaptations are given leading to potential variability in interpretation. However; uniformity in the management of ALS concerning these aspects is in the interest of the patient with ALS.

Objectives: To investigate current practice of the specialized ALS care teams with respect to the timing of PEG, respiratory care and aids and adaptations.

Methods: A structured questionnaire was sent to the rehabilitation specialist being the representative of the ALS care teams.

Results: Thirty-nine (86%) of the care teams returned the questionnaire. The results showed that there is large variation in care regarding:

- PEG: Sixty-two percent of the teams use decline of respiratory function as a criterion to discuss PEG. Thirteen percent do not use weight loss as a criterion to discuss PEG. Forty-four percent discuss PEG at progressive dysarthria. In about 50% of the teams PEG insertion takes place during a 3-day hospitalization; in the remaining teams, in a daycare setting. The team member who informs and prepares a patient for PEG varies widely.
- Respiratory care: all teams discuss the possibility of NIPPV at some point in disease progression. When nocturnal hypoventilation is present 74% of the teams discuss the possibilities of ventilatory support. Progressive dysarthria is 29% of the teams' reason for discussing the possibilities of ventilatory support. Forty-three percent never or incidentally monitor a patient's vital capacity.
- Aids and adaptations: 48% of the teams reported that aids and adaptations are realised late or too late. Apart from patients' delay which is also a significant factor, two-thirds of the teams stated that local government is an impeding factor.

Discussion and conclusions: The results showed that there is no uniformity in the management of ALS patients with regard to PEG, respiratory care, and aids and adaptations. The Dutch guideline lacks details on these subjects. As a first step towards nationwide consensus regarding the timing of interventions in ALS, a national symposium was held in January 2005 to communicate and discuss the results of this study. The ultimate goal is to improve and standardize care of ALS patients in the Netherlands according to an evidence-based guideline.

P168 DO ALS PATIENTS CARRY OUT PHYSIOTHERAPY SPECIFIC RESPIRATORY MUSCLE TRAINING CORRECTLY?

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Background: Impairments related to ALS are defined by strength loss, respiratory insufficiency, spasticity, loss of fine motor coordination, speech and swallowing difficulties. Physical therapy can potentially maximize muscle and respiratory function prolonging survival, independence in activities of daily living and quality of life. This study forms part of a larger study 'Clinical Practice Evidence- based Guidelines for Patients with ALS/MND'.

Objectives: The purpose of the study was to determine whether ALS patients carry out physiotherapy specific respiratory muscle training correctly.

Methods: Observational prospective study. The inclusion criteria were: diagnosis of probable or definitive ALS (El Escorial diagnostic criteria). ALS patients from Hospital Carlos III, Madrid, Spain were included in the study.

The 'Clinical Practice Evidence- based Guidelines for Patients with ALS/MND' include specific respiratory muscle training in the home. To determine the correct realization of the home exercises, two steps were carried out: in a first session, the patient learned and executed the exercises under the supervision of the physiotherapist; and in a second session (a month later) the realization of specific respiratory muscle training was determined without help of the physiotherapist.

Results: Twenty patients were assessed in the study. Only 20% correctly carried out all the programme of physiotherapy specific respiratory training a month after training. Seventy percent of the patients did not know that they were performing the exercises incorrectly. Thirty percent had doubts on the accomplishment.

Conclusions: ALS patients do not perform physiotherapy specific respiratory muscle training correctly. In a high percentage (80%), the execution is poor and in 70% of cases the patient does not know they are performing the exercises poorly. Therefore, periodic supervision by the physiotherapist is recommended to ensure patients carry out the respiratory exercises correctly and efficiently.

P169 RESPIRATORY AND NUTRITITIONAL SUPPORT: A QUALITATIVE STUDY OF EXPERIENCES OF ALS PATIENTS

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Background: Patients with ALS all encounter severe problems with eating and breathing when the disease progresses. To reduce these problems, patients have the possibility to use respiratory and/or nutritional support. Some research has been done into the survival of patients using this kind of support and the effects on quality of life of these patients and their carers. These studies all used a quantitative methodology. To our knowledge no qualitative studies are available concerning the experiences of ALS patients with respiratory and nutritional support, and how they experience their body when non-invasive ventilation or a percutaneous endoscopic gastroscopy (PEG) is administered and is of life-saving importance.

Objectives: 1) To explore the experiences of ALS patients who use respiratory and/or nutritional support (non-invasive ventilation and/or a PEG) for at least three months and to focus on changes in body perception; 2) To improve the instructions given by the professional team to the ALS patient and their carers on respiratory and nutritional support and to give optimal care/guidance if patients choose and use the support; and 3) To stimulate a discussion about the ethical implications of the nutritional and respiratory support for the patients.

Methods: Nine patients (seven females, two males) with ALS who used respiratory and/or nutritional support for at least three months, were interviewed at home twice. A semi-structured in depth interview guide was used. All interviews were recorded on tape and transcripts were made. Transcribed interviews were analysed using the constant comparative method. Facts about disease onset, family and home situation etc., were assessed with a questionnaire prior to the interview. Functional status was assessed using the ALS Functional Rating Scale-Revised. All patients were treated in the ALS Centre of the Academic Medical Centre of Amsterdam (AMC).

Results: Preliminary results from the constant comparative analysis revealed that the choice for respiratory and nutritional support is, for ALS patients, often not a choice;

they feel obliged to choose the intervention in order to avoid regret afterwards. The influence of ALS, non-invasive technology and PEG on body perception is diverse. The body is perceived as a burden and a source of shame because of dependence on others and functional impairment. People with bulbar ALS reported feelings of shame in social life. The technology has influences on the perception of the body and intimate relations with others.

Conclusions and discussion: This qualitative research described the experiences/stories of patients with ALS using respiratory and/or nutritional support. The authors hope to stimulate a discussion about the value of qualitative research in the care for ALS clients.

P170 THE ISSUES ON THE INDUCTION OF INVASIVE MECHANICAL VENTILATION IN JAPANESE ALS PATIENTS

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Background: In Japan, about 30% of ALS patients choose to use invasive mechanical ventilation (IMV). This figure is much higher than that of western countries. We may assume that one of the reasons is the Japanese way of thinking about death. We performed this study to investigate what proportion of healthy Japanese people would choose to live on IMV, if they developed ALS.

Objective: To investigate what proportion of the healthy Japanese population would choose to live on IMV if they had ALS.

Method: A questionnaire-based (Q1,Q2) study about the induction of IMV was performed on 89 medical students before and after clinical training (which included a visit to ALS patients living on home mechanical ventilation), and 25 neurologists at our institute.

Q1: If you were an ALS patient, would you choose to live with IMV?

Q2: Would your decision be different if you were able to withdraw from IMV at your own request?

Results: Among students before clinical practice 43.4% chose the induction of IMV; the figure dropped to 31.1% after clinical practice (they observed advanced ALS patients). Only 16.7% of neurologists chose IMV. If they could withdraw from IMV, 54.5% of students and 58.3% of neurologists chose induction of IMV.

Discussion and conclusions: Based on our results we assume that knowing more about ALS made students less inclined toward the induction of IMV. The percentage of induction of IMV in Japan is 30%. This figure is almost the same as the response of the students after clinical practice. Thus if patients know the situation after induction of IMV better, the figure might drop to the same level as neurologists. However, even the neurologists' response that 17% chose to wear IMV is a remarkably higher percentage compared with that of western countries. Thus, the Japanese population might have different ways of thinking about death.

The decision to choose IMV becomes more than 50% among both students and doctors if they can withdraw from IMV. In western countries the right of withdrawal from IMV is usually permitted, yet only a small percentage of patients with ALS chose IMV. In Japan the cost of IMV is covered by health insurance, so patients do not need to consider the economic burden so much. This may be one of the main reasons why so many more patients choose IMV in Japan. However, the majority of patients still do not chose IMV, because they may think they cannot accept the advanced stage of ALS and the totally locked-in state, and also because of social reasons such as difficulties of caregivers.

P171 THE COST-EFFECTIVENESS OF EARLY NON-INVASIVE VENTILATION FOR ALS PATIENTS

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Background: Optimal timing of non-invasive positive pressure ventilation (NIV) initiation in patients with amyotrophic lateral sclerosis (ALS) is unknown, but NIV appears to benefit ALS patients who are symptomatic of pulmonary insufficiency. This has prompted research proposals of NIV initiated earlier in the ALS disease course in an attempt to further improve ALS patient quality of life, and perhaps survival. Prior to initiating clinical trials of novel treatments, the potential cost-effectiveness of the treatment should be considered. Cost-utility analyses allow for estimation of the cost-effectiveness of treatments in relation to their effects on quality of life, a very important outcome in ALS studies.

Objectives: We used a cost-utility analysis to determine a priori what magnitude of health-related quality of life (HRQL) improvement early NIV initiation would be necessary to be cost-effective in a future clinical trial. We reasoned that should the degree of improvement determined in this analysis seem plausible, future clinical trials testing early NIV would be warranted from an economic perspective. If, on the other hand, the analysis showed that an impractical degree of improvement would be necessary for the treatment to be cost-effective, future clinical trials of early NIV for ALS would be less worthwhile.

Methods: Using a Markov decision analytic model we calculated the benefit in health-state utility that NIV initiated at ALS diagnosis must achieve to be cost-effective. The primary outcome was the percent utility gained through NIV in relation to two common willingness-to-pay thresholds: $50,000 and $100,000 per quality-adjusted life year (QALY).

Results: Our results indicate that if NIV begun at the time of diagnosis improves ALS patient HRQL as little as 13.5%, it would be a cost-effective treatment. Tolerance of

NIV (assuming a 20% improvement in HRQL) would only need to exceed 18% in our model for treatment to remain cost-effective using a conservative willingness-to-pay threshold of $50,000 per QALY.

Discussion and conclusions: If early use of NIV in ALS patients is shown to improve HRQL in future studies, it is likely to be a cost-effective treatment. Clinical trials of NIV begun as early as the time of ALS diagnosis are therefore warranted from a cost-effectiveness standpoint.

P172 CHARACTERISTICS OF HOSPITALIZATIONS IN AMYOTROPHIC LATERAL SCLEROSIS BETWEEN 2000 AND 2002 BASED ON A US NATIONWIDE SAMPLE

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Background: Patients with amyotrophic lateral sclerosis (ALS) had lengthy hospitalizations with high mortality rate in a US study for the year 1996 (1). We had previously evaluated a smaller dataset, the National Hospital Discharge Survey (NHDS) and found no significant trends in hospitalization demographics and outcomes for 1996–2002. Given that ALS is relatively rare, the NHDS dataset (ca. 270,000 records/year) captures relatively few ALS hospitalizations. We are now reporting findings based on the Nationwide Inpatient Sample (NIS, ca. 7,000,000 records/year).

Objective: To study the demographics and expenditure in hospitalizations of ALS patients between 2000 and 2002 based on the NIS.

Methods: We used the NIS, a large all-payer US inpatient care database containing data from about 1000 hospitals, approximating a 20% stratified sample of US hospital discharges. We included patients ≥ 18 years of age. Cases were identified by ALS diagnosis (ICD code 335.20) and compared to hospitalized subjects without the ALS diagnosis. Continuous variables were compared by *t*-test, and categorical variables by Pearson's χ^2 test.

Results: The number of ALS hospitalizations relative to the total US hospitalizations between 2000 and 2002 has been stable (22.8 to 23.2/100,000). Hospitalized ALS patients were significantly older than non-ALS patients throughout the survey period ($p < 0.01$). They were more likely to be men than non-ALS patients ($p < 0.01$). The racial and ethnic distribution was significantly different comparing hospitalized ALS from non-ALS patients ($p < 0.01$). In all years surveyed, ALS patients were more likely to be white and less likely to be black or Hispanic. The mean length of stay was twice as long for ALS patients (8.1–8.32 days) than for non-ALS patients (4.5–4.7 days). ALS admissions were more likely to be classified as emergency admissions ($p < 0.01$) and associated with high inpatient mortality ($p < 0.01$). These findings remained unchanged between 2000 and 2002. Mean hospital stay expenditure increased from 2000 to 2002 by 27% to $ 17,455 in non-ALS and by 44% to $ 33,683 in ALS patients.

Discussion and conclusions: Admissions in ALS patients continue to be predominantly emergency admissions. They are associated with above-average length of stay and high in-hospital mortality. The disproportionate increase in expenditure for ALS hospitalizations compared to non-ALS hospitalizations is not explained by changes in length of stay. In addition to the well established age and gender disparities between ALS and non-ALS patients, we found consistent racial and ethnic disparities in all years surveyed. Hospitalized ALS patients are more likely to be white and less likely to be black or Hispanic compared to non-ALS patients. Whether this disparity stems from socioeconomic, patient, or other factors remains to be determined.

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P173 ALS PATIENTS' CARE: THE MND CLINIC OF MONTPELLIER. EXPERIENCE OF A MULTIDISCIPLINARY APPROACH

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Background: ALS is a disorder with a severe prognosis, inducing rapid and significant motor handicap. A rapid diagnosis, a systematic and complete approach for the management of the disease is needed and is expected by both the associations and the patients.

Objectives: To describe the organisation of our MND clinic. To present the data from one year (2004) in terms of clinical management and patient care.

Methods: The MND clinic, as 17 others in the country, was labelled by health authorities in 2003. The goals are: 1) precocious diagnosis; 2) systematic and homogeneous patient management; 3) organization of home care; 4) formation of care workers throughout the region; 5) organization of research. The staff is composed of three neurologists, two nurses, one psychologist-neuropsychologist, one occupational therapist, one social worker, one secretary, one technician (evaluation of the patients), and two clinical research assistants.

Results: A total of 320 patients, of which 120 were newly diagnosed, are being followed in the clinic. This represents a total of 1200 medical consultations, as the majority of the patients are monitored quarterly. The clinic is tightly

connected with the neuromuscular unit. False positive and false negative diagnoses of ALS remain the same between 2003 and 2004. The delay in diagnosis ranges between one week (bulbar case with dysphonia) to four years (respiratory onset with early tracheostomy and ventilation). Median survival is 48 months. Twenty percent of the patients have more than five years of evolution. Shorter survival was six months.

ALS evolution is regularly evaluated by spirometry, blood gases, weight, impédancemétrie, oxymetry, nutritional state, together with muscular testing and ALSFR-S. Five hundred consultations have been completed for both the occupational therapist and the psychologist (including 40 comprehensive cognitive evaluations). The specific case of more than two-thirds of the patients was considered by the social worker, followed by a social intervention in more than 50 cases. Research activity consists of the following programmes: 1) familial ALS network; 2) risk factors and hypoxia; 3) proteomics; 4) epidemiology and environmental risk factors; 5) cognition; 6) therapeutic trials and academic programmes (more than 150 patients included).

Discussion and conclusion: The Montpellier MND clinic is the second largest in the country. The rather young organisation has not yet permitted the development of protocols for care, information and a regional network for ALS care. These are our main goals for 2005–2006.

P174 MULTIDISCIPLINARY ALS CARE IMPROVES QUALITY OF LIFE IN PATIENTS WITH ALS

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Objective: To examine the effect of multidisciplinary ALS care on the quality of life (QoL) in patients with ALS and their caregivers.

Methods: In a cross-sectional study 208 patients with ALS and their caregivers were interviewed. QoL was assessed using the 36-item Short Form Health Survey (SF-36) and two visual analogue scales (VAS). Criteria for multidisciplinary ALS care were: 1) an ALS team headed by a consultant in rehabilitation medicine and consisting of at least a physical therapist, occupational therapist, speech pathologist, dietician and a social worker; 2) use of the Dutch ALS consensus guidelines for ALS care; and 3) at least six incident ALS patients per year.

Results: Clinical characteristics and functional loss of the 133 patients receiving multidisciplinary ALS care and the 75 patients receiving general ALS care were similar. The percentage of patients with adequate aids and appliances was higher in those with multidisciplinary ALS care (93.1 vs. 81.3 %, $p=0.008$), while the number of visits to professional caregivers was similar in both groups. Patients in the multidisciplinary ALS care group had a better mental QoL on the SF-36 Mental Summary Score than those in the general care group ($p=0.01$). The difference in QoL was most pronounced in the domains of social functioning and mental health, and was independent of the presence of aids and appliances. No significant differences were found in the SF-36 Physical Summary Score, VAS, or in QoL of caregivers of patients with ALS.

Conclusion: High standard of care improves mental QoL in patients with ALS.

P175 LIVING WITH ALS/MND: MAINTAINING PERSONAL INTEGRITY IN THE FACE OF ONGOING CHANGE AND ADAPTATION

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Background: The progressive death of motor neurons causes difficulties with mobility, communication, breathing and nutrition for people diagnosed with ALS/MND. While most research focuses on the disease progress, little is known of the illness experience.

Objectives: The aim of this study was to explore and describe what it is like to live with ALS/MND and identify how people with the disease negotiate with others for their choices in life. The purpose was to give people with the disease a rarely heard voice in qualitative research.

Method: A grounded theory approach was used to explicate the life world of people diagnosed and living with ALS/MND. Sample size was 25 people with the disease from rural and urban areas. Data sources were 33 transcribed in-depth interviews; written stories, prose, songs and books important to the people; and researcher field notes. The data were analysed using constant comparison analysis and managed with the software program N-Vivo.

Results: Analysis revealed a story of being diagnosed that was integral to understanding the illness experience of ALS/MND. Living with the disease involved the development of a decision-making process to cope with the ongoing changes that emanated from the disease's progression. The pattern was cyclic and repetitive. The basic social process that underpinned the decision-making while living with the disease was that of maintaining personal integrity. Living with ALS/MND was revealed as maintaining personal integrity in the face of ''ongoing change and adaptation''. Facets of personal integrity were feelings, image and control. Strategy types for maintaining personal integrity were protecting, regaining and sustaining. Contextual aspects that influenced the processes were past experiences of the health care system and

socioeconomic factors. The threats participants experienced as they endeavoured to maintain personal integrity were impaired communication, ''bad days'', and health professionals and service providers. Overall, participants said it was ''hard work'' to live with ALS/MND.

Discussion and conclusions: Laughing, denying, withdrawing and thinking creatively were important tactics used in maintaining personal integrity, but such strategies were mostly misunderstood by, or invisible to, the nurses and health care professionals who cared for the participants. As such this study revealed the hidden ALS/MND illness experience, which until now has been unacknowledged and not included in care management plans. Implications are that better understanding by health professionals of the hidden experiences will enable them to facilitate sensitive, supportive and innovative care of people with ALS/MND and their families.

Acknowledgement: This project was funded and supported by The Australian Research Council, Deakin University, Bethlehem Griffiths Research Foundation, Calvary Health Care Bethlehem Inc and the Motor Neurone Disease Association of Victoria. Susan King was a joint recipient of the Nina Buscombe Award 2002 and 2004.

P176 PERSONAL EXPERIENCES OF LIVING WITH ALS/MND: ILLNESS NARRATIVES POSTED ON THE ''WEB''

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Background: Personal illness narratives provide powerful insights into how individuals adjust to and cope with illness. Such narratives can be obtained directly from patients, from traditionally published accounts and more recently from unpublished personal stories posted on the internet. This paper reports on one part of a study investigating published and unpublished personal narratives on ALS/MND. To date no published work has sought to investigate internet based writing about life with ALS/MND or to determine who is using this medium to publicise their illness narratives. Using the internet as a source for personal narratives presents the researcher with challenges regarding how to track down relevant information and make sense of the volume of potential material available.

Objectives: This paper describes the processes undertaken systematically to locate unpublished personal narratives written by people with ALS/MND posted on the internet and to identify the characteristics of the authors to establish who is using this medium to tell the story of their illness.

Methods: A systematic electronic search was carried out between January and March 2005 to identify and locate internet based narratives written by people with ALS/MND. Numerous search terms were employed to ensure that commonly recognized nomenclatures for the disease were incorporated. A pathway approach for the search allowed links between sites to be followed up until all links were checked and saturation was apparent. Following the application of inclusion/exclusion criteria 86 sites were accessed, from which personal writings were downloaded, archived and subject to content analysis.

Results: Substantially more males (76%) than females (24%) posted personal ALS/MND narratives on the internet. North America (USA 59%, Canada 21%) produced more narratives than Australia/New Zealand (11%) or Europe (9%). Authors came from a variety of occupational backgrounds. The largest occupational group (27%) consisted of manual or unskilled workers; the second largest occupational group (19%) comprised armed forces/emergency services; professional writers/academics, people from the film and music industries and health care professionals each produced 8% of the narratives. Fifty-eight sites included the author's age at diagnosis (range 20–67 years); a majority (54%) of authors were under 40 years of age at the time of their diagnosis. The experiences reported spanned the decades from the 1960s, but most authors (58%) received their diagnosis during the 1990s. Most narratives (58%) were restricted to a single entry in one section of a personal website; many (30%) were restricted to a single webpage; ongoing multiple journal entries were found on 15% of sites.

Discussion: This novel study demonstrates that researchers can access an invaluable source of personal narratives using a systematic approach and shows the extent to which a wide cross-section of people with ALS/MND have used new technology to publicise their personal stories.

P177 BUILD: A VIRTUAL SUPPORT NETWORK FOR PEOPLE WITH MND

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Background: Communication difficulties, distance, social anxieties, and other commitments may make it difficult for some people with MND to attend 'real-world' support groups such as local support group meetings.

Objectives: To develop an online community for people with MND, their carers, and healthcare professionals to support one another, exchange advice, debate alternative therapies, and encourage public awareness about the condition.

Methods: Since 2001 a virtual message board forum has been established that is free to access for anyone

with an internet connection (www.build-uk.net) Since 2002 there has been a weekly real-time chat room for members to communicate in a safe environment. In 2005 the first Greek-language MND forum on the internet was established for use by our Greek-speaking visitors.

Results: Currently the site has 117 members, of whom 25 are 'regular contributors'. In the past two years the established regulars have become a crucial source of emotional support for people affected by MND including unaffected individuals at risk of inheriting the familial form of MND. One regular contributor said: ''At my local MND Association meetings, there aren't many people with MND that go. So BUILD gives me the chance to converse with other sufferers, not only from the UK but from all over the world. Also the relative anonymity afforded by a forum like BUILD allows us to discuss sensitive subjects which we would probably never discuss with anyone face to face. I think of BUILD as my internet home and the people here as my extended family.'' Another regular contributor said ''I am the youngest person at our (local) meetings and coming here means I can talk to people nearer my age as well.'' Storage of previous discussions provides an archival database of material for newly-diagnosed patients to learn more about their condition and become 'expert patients'.

Conclusion: The increasing use of the internet by all sectors of the MND patient population means that information and support will increasingly be delivered by high-tech means in the coming decade. The availability and anonymity of the internet means that patients are able to discuss sensitive subjects in a safe environment. Online systems also provide convenient methods of data collection and potentially clinical monitoring. Future developments may include secure virtual forums for health care professionals to form collaborations and discuss research.

P178 INTRODUCING COMPUTER ACCESSIBILTY OPTIONS TO INDIVIDUALS WITH ALS

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Background: New technologies are giving individuals with motor disabilities alternative access to their computers. Information regarding these technologies is available from a variety of sources. Patients are not always aware of these sources.

Objective: To assess the patients' knowledge of computer accessibility in order to determine if there is a need to provide education in this area.

Methods: A questionnaire was administered to our clinic patients to assess their computer requirements and any difficulties they may find accessing their personal computers. They rated any difficulty they were having with their computer keyboard and mouse. Additionally, they rated their knowledge of the accessibility features currently available on the computer, as well as the software or hardware that can assist in their computer use. Information was collected on how the patients have gained their existing knowledge and what they think the proper venue to obtain future education would be. We recorded the ALSFR-S score and the patient's forced vital capacity score. In addition, the individuals rated their quality of life on a single item scale. The data were analyzed using the t-test ($p=0.05$).

Results: A total of 18 patients completed the questionnaire. Ten indicated the place to learn of computer accessibility technology would be from a health professional. We broke the data down by ALSFR-S score, specifically looking at hand function. Nine patients scored a 0 or 1 in the ALSFR-S category of handwriting. Of those individuals, seven indicated health professionals as the preferred source of information ($p=0.05$).

Discussion and conclusions: Our patients were reporting they would stop using their computers altogether as they began to have difficulty with access. Information is available on computer accessibility, but finding it appeared inconsistent. From this study we have learned that patients would like to receive computer accessibility information from their health professionals, yet most have relied primarily on finding it themselves. This is an unfulfilled need that the therapists can address with their patients. As a direct result of our initial survey, we have developed an educational program to address this need. This includes education on available equipment, as well as providing a trial of equipment at the clinic for interested individuals.

P179 USE OF MODERN COMMUNICATION TECHNOLOGY BY PATIENTS WITH MOTOR NEURON DISEASE IN NORTHERN IRELAND

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Background and objectives: Modern communication technologies open up novel ways of managing motor neuron disease (MND) either by providing information about the disease and its symptoms by e-mail or on a website, or indeed by connecting the doctor to the patient using e-mail or video conferencing.

Whether this is feasible depends on how available these technologies are in patients' homes. To find this out we used the Motor Neurone Disease Register in Northern Ireland to ask patients by telephone a number of questions about how available modern communication technologies were to them.

Methods: The survey was carried out between March and May 2005. A questionnaire was sent to all 78 people on the Northern Ireland MND Register which aimed to find out information on the use of an extra telephone line, broadband or integrated services digital network (ISDN).

We asked how many people had fax machines or a computer at home and how many used e-mail and internet in general and whether they used them specifically to find information on MND. We asked them also how many thought that their relatives had found information on MND using the internet.

Results: Eighty-five percent of people returned the questionnaire. Of those who replied 10% had broadband or an extra line, 4% had a fax machine, 1% an ISDN line. Thirty-six percent possessed a computer with 27% using it for e-mail and 30% for the internet. Thirteen percent of people had used e-mail in connection with MND and 19% the internet. Forty-seven percent felt that their relatives had used the internet to find out about MND.

Conclusions: Use of e-mail and internet is remarkably low among patients with MND in Northern Ireland. Similarly, less than half of all patients' relatives had used the internet to find out information about the MND in their family. Northern Ireland has been one of the first places to make broadband universally available so there is no reason to think it is technologically backward. It is unlikely that the Motor Neurone Disease Association can increase the number of patients who are internet users and so it will need to continue to provide much of the information about MND in written form. Also, if telemedicine is to be used to deliver care to patients with MND at home, the necessary communication infrastructure will need to be introduced which will increase the cost.

P180 QUEST (QUEBEC USER EVALUATION OF SATISFACTION WITH ASSISTIVE TECHNOLOGY) WITH COMMUNICATION AID USERS: A PILOT TRIAL IN THE UK

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Background: QUEST (Quebec User Evaluation of Satisfaction with assistive Technology) is an outcome measure that can give assistive technology users a way of expressing their level of satisfaction in a structured way. QUEST is a useful tool to assistive technology practitioners to help assess the usefulness of prescribed assistive equipment.

QUEST has been successfully validated for mobility products and daily living technologies but not for Electronic Assistive Technology (EAT). EAT includes Augmentative and Alternative Communication (AAC) (also known as Communication Aids) and Environmental Controls.

Objectives: The aim of the pilot study was to examine the views of experienced high-tech AAC device users (n=8). The pilot looked at how suitable QUEST 2.0 is for Communication Aid users and if modifications to QUEST 2.0 are needed for future use.

Methods: Participants were experienced AAC users between the ages of 18 and 65 years. All participants were able to give informed consent agreeing to participate. Participants were Communication Aid users with a variety of conditions; motor neuron disease (n=6), cerebral vascular accident (n=1) and cerebral palsy (n=1). Participants used a LightWRITER (n=7) or an adapted HP Jornada (n=1).

Results: Participants were broadly 'quite satisfied' with their Communication Aids. However, the majority had an area where they seemed to be less satisfied. Some questions prompted further comments and others appeared to be non-applicable to participants.

Discussion and conclusions: QUEST proved to be a quick, effective outcome measure that was easy to administer. The use of comments gave useful, if subjective information. Non-applicable responses also gave interesting feedback.

QUEST 2.0 appears to be a useful instrument for an AAC professional examining the satisfaction of their clients with a Communication Aid prescription. Further work to formally validate QUEST 2.0 for AAC and EAT in general is therefore desirable.

P181 RESEARCH ON COMMUNICATION WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS IN A TOTALLY LOCKED-IN STATE: INTERVIEWS WITH CAREGIVERS

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Background: Depending on the severity of the disease, an artificial respirator can maintain life in ALS. However, as the disease progresses, paralysis of all voluntary muscles including those of the eye occurs, resulting in a totally locked-in state (TLS). At present, a device that can differentiate affirmative and negative responses based on EEG or cerebral blood flow is available. However, training to utilize this type of device is difficult, and only a handful of patients can communicate through such mechanisms in Japan.

Objective: The objective of the present study was to gather cues to assist in communication with patients with severe communication disorders by interviewing caregivers of ALS patients in TLS.

Methods: A semi-structured interview was conducted with six caregivers of four ALS patients in TLS, and the contents of the interview transcripts were categorized in order to extract related factors.

Results: Communication cues included such physical reactions as tachycardia, increased blood pressure, facial

flushing, and perspiration. Caregivers interpreted these reactions as signs of tension, anxiety, fear, and pain, and also attributed importance to eye positions and slight eye movements. Caregivers believed that patients were asleep if their eyes were in a superior gaze position. Moreover, they stated that although patients' eyes did not move in response to normal questions and interactions, they were sure that either the eyes moved very slightly over a period of time or that the lower eyelid contracted in response to important questions regarding therapy or social activity. Caregivers interpreted the volume of lacrimation, salivation, and tracheal secretion as indicators of happiness, sadness, and anxiety. Caregivers believed that they could read facial expressions to indicate peacefulness, laughter, and strong affirmation based on gaze; however, since these signs lack objectivity, caregivers did not base their care on their instinct and intuition.

Discussion: Due to limited verbal communication from patients, caregivers focused on non-verbal communication cues to ascertain emotions and patiently observed eye movements in an attempt to communicate.

P182 NEW PARTNERSHIPS TO IMPROVE THE QUALITY OF LIFE OF PEOPLE WITH MOTOR NEURON DISEASE AND THEIR CARERS

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Background: Motor neuron disease (MND) has become established as a target population for palliative care services. The involvement of appropriately educated palliative care volunteers is a vital part of integrated care pathways and has the potential to reduce the acute sense of isolation resulting from MND. The usual practice of introducing volunteers during the later stages of MND can be problematic, particularly when communication difficulties impede the establishment of a constructive relationship. A new approach to palliative care volunteers is therefore required, to facilitate optimal access for people with MND and MND specific education for volunteers.

Objectives:

- To maximize satisfaction and well-being for both the client and the volunteer.
- To introduce comprehensively and appropriately educated volunteers to clients in the early stages of MND.
- To provide structured peer support and professional supervision to volunteers.
- To develop partnerships with existing palliative care volunteers

Methods: A MND volunteer visitor pilot programme was initiated. Focus groups and discussions with palliative care services helped to develop guidelines for volunteer recruitment, education and support. Existing MND information was adapted for a volunteer education programme and manual and ethical approval was obtained. Suitable MND volunteers were recruited, screened and educated and existing palliative care volunteers were invited to participate in the education programme. A range of questionnaires was developed for clients, carers and volunteers to establish the value of the education programme and the subsequent visits. People living with MND and their carers were interviewed to facilitate appropriate placement.

Results: The volunteers evaluated the education programme positively. A comprehensive education programme and manual for volunteers has been developed, with CD and facilitator's kit. MND volunteers have been placed with people with MND. Appropriate supervision has been provided for volunteers as well as regular peer support meetings. Evaluations of placements have been varied.

Conclusion: Volunteers are an important part of the integrated care pathway for people with MND. Volunteers can help people with MND achieve goals and therefore enhance quality of life. Appropriately educated volunteers may also provide a crucial link between the health care system and people with MND. This new approach provides the opportunity to introduce MND volunteers in the early stages and develop partnerships with existing palliative care services to facilitate the provision of MND specific education for palliative care volunteers.

P183 SELF-DETERMINATION BY PATIENTS WITH ALS/MND IN JAPAN: A PRELIMINARY SURVEY

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Background: In Japan, a quarter of patients with amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND) have been put on a mechanical ventilator with tracheostomy (TMV) at the advanced stage of the illness (1). Hayashi et al. (2) reported that 18% of patients with TMV had advanced to a totally locked-in state (TLS) at the end of the course. Currently, Japanese law prohibits anyone (including the attending physician) from discontinuing assisted ventilation once it has been started, even if the patient wishes to do so. Although there has been a lack of discussion on the way one dies in this situation, there has been considerable media information on the condition of ALS/MND at the advanced stage that may make the patients anxious, to the extent that they have concerns about their self-determination. To our knowledge, there is no published report concerning the autonomy or the self-determination of patients with ALS/MND in Japan or Asia.

Objective: To study the patients perception of his or her autonomy concerning options of management at the advanced stage of ALS/MND.

Methods: Semi-structured interviews were conducted at the homes of 14 patients with ALS/MND in the Mie prefecture of Japan. Issues explored during interview included: 1) the patients' perception of self-determination or autonomy; 2) the patients' thoughts about expressing such self-determination. These interviews, which also covered diagnosis, physical function and circumstance of care at home were carried out during the visiting survey from November 2004 to March 2005 by one of the authors (YN).

Results: Fourteen (10 males and 4 females) of the 21 patients surveyed were eligible for analysis on the basis of diagnosis, communication ability and cognitive function. Mean age was 57.9 years ($\pm$12.0). Nine patients seemed to poorly understand 'autonomy' ('jiritsu' in Japanese). Eleven patients answered that they wanted to exercise their right of self-determination, while the degree by which the self determination should be respected (on a percentage scale) was 89.1 ($\pm$17.6) ranging from 50 to 100%.

Conclusions: Many patients suffering from ALS/MND considered self-determination to be important, to choose the options for the medical management at the advanced stage of their diseases, while 'autonomy' was still unfamiliar to some Japanese patients.

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P184 ALS PATIENTS RECEIVING HOSPICE CARE

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Background: The hospice is an integral aspect of ALS patient care during their end-of-life experience. Can it be assumed that hospices are familiar with the needs of ALS patients?

Objectives: We developed a questionnaire based on our experience with ALS patients at their end of life to determine how familiar hospice services are with these issues.

Methods: One hundred and fourteen sites were chosen from the National Hospice and Palliative Care Organization (NHPCO) website @ www.nhpco.org; one urban and one rural site from each state except the surrounding states of Utah where 4–6 sites were selected. Initially questionnaires were mailed, but when less than 10% were returned the questionnaires were resent by fax. When this resulted in only an additional 5% return rate, random calls to 10 hospice agencies resulted in four more returns. Most returns were incompletely filled out.

Results: Of 21 returns from 114 questionnaires sent, 30% reported no ALS patients in 2004, and only two agencies saw more then 15 ALS patients in 2004. Length of service (LOS) ranged from five to 232 days, compared to other diagnoses where the LOS ranged from one day to 547 days. Ten respondents felt their knowledge base of ALS was the same as other disorders; six felt it was lower and one felt it was higher as they had a registered nurse who saw all of their ALS patients. There were 16 responses to the question of whether there were cognitive changes in ALS patients and 11 said they did not see any cognitive changes and five responded "yes". The greatest challenge for hospice workers was communication with ALS patients, and the second greatest challenge was providing support to the caregiver. Equipment needs of ALS patients outweigh equipment needs of patients with other diagnoses, but clinical services offered to both groups are comparable. Symptoms consistent with upper motor neuron involvement are more prevalent in the ALS population in hospice but all other symptoms seem to be equally seen in both ALS and other diagnoses. Seventeen respondents were not aware of the American Academy of Neurology Practice Parameters, 'Care of the Patient with ALS'. One agency was aware of the standard but did not actively follow the recommendations.

Discussion: We were surprised at the low response rate, despite different attempts to obtain completed questionnaires. Based on the responses, hospices in general appear to have little experience with ALS patients. ALS versus other diagnosis is a small percent of the number of patients cared for in hospice and therefore there is no initiative to gain greater knowledge in this devastating disorder.

Conclusion: The ALS community needs to be more proactive in educating hospice agencies in the care of patients with ALS. Distributing the Practice Parameters through National Hospice Organizations is a good start. Providing educational pamphlets about the disorder and presenting at hospice conferences would help educate providers about the needs of ALS patients and maximally help them through their process of dying.

P185 MOTOR NEURON DISEASE: PALLIATIVE CARE FROM THE ONSET

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Background: Within the acute setting the Hospital Palliative Care Team (HPCT) identified an unmet need for support and access to specialist palliative care services for MND patients and their carers.

Objectives: By developing and integrating our service with the existing MND service we aimed to facilitate access to local specialist palliative care services and

enhance the quality of care received by patients and their carers.

Methods: Following formal discussions, a Macmillan nurse attended a weekly MND assessment clinic (two sessions) receiving referrals for first follow-up and specific support needs from July 2002. A year later this service was increased due to the demand and complexity of referrals to three outpatient sessions involving two Macmillan nurses. This provides contact from the time of diagnosis. To date we now see all MND patients attending all three sessions. Throughout, inpatient assessment for support, symptom management and discharge planning has occurred, alongside defined telephone support for outpatients and carers. In addition the HPCT has a computer alert system informing us of readmissions to the acute setting facilitating a more rapid response from the MND team and HPCT.

Results: The table demonstrates access to the HPCT

	Aug 2000 – Jun 2002	Jul 002 – Mar 2004	Apr 2004 – Apr 2005
New diagnosis	0	66	73
Established disease	3	46	93
Inpatient contacts	2	49	31
Outpatient contacts	5	181	306
Referrals onto specialist palliative care services	1	43	30

Discussion and conclusions: From the patient and carers' perspective the introduction of palliative care from the onset has been welcomed. Significant issues are discussed in a proactive and timely manner with the aim of empowering the patient, focusing on the patient's agenda and initiating thoughts of the challenges ahead. The primary care teams follow up these discussions, with access to further specialist palliative care services if needed, providing a continuity of service for the patient and improved communication between the settings. Interdisciplinary team working has developed, improving cross boundary working and communication across health, social and voluntary care professionals and settings. Development of support networks and education is evolving from this model of service delivery alongside openness and respect of roles.

P186 END OF LIFE CARE DECISIONS IN ALS: A CROSS-ETHNIC PERSPECTIVE

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Background: Death and dying are extremely profound events that bring into focus important ethical, cultural, religious, and medical issues to all patients. ALS leads to progressive and often predictable decline in motor function, respiratory failure and death, while mental capacity and intellect generally remain intact in all patients. It is not known if the end-of-life care decisions differ in patients with ALS from different ethnic groups in a multi-ethnic society.

Objective: To study end of life decisions and clinical practices in an ethnically diverse population of patients with ALS.

Methods: We prospectively followed 66 patients (39 males and 27 females) of diverse ethnic groups with end-stage ALS who were seen at the Kessnich Family MDA-ALS Center and died between January 2002 and June 2004. Information with regard to patient advanced directives, health care surrogates, ALS care choices, hospice use, and palliative care was collected.

Results: The cohort of patients comprised 41 white Caucasians (W, 62%), 19 Hispanics (H, 29%) and six African Americans (A, 9%). Most patients anticipated death and died peacefully (93.9%) in this group. At the time of their death, 32% (W 36.6%, H 26.3%, B 20%) of them had feeding tubes, 70% (W 70.7%, H 78.9%, B 33.4%) were on non-invasive ventilatory support, and 7.6% (W 3%, H 15.5%, B 0%) had a tracheostomy and permanent ventilatory support. Advanced directives (with or without surrogate designees) were in place in 92.4% (W 95.1%, H 89.5%, B 83.3%) and were followed in 96.7% (W, 97.5%, H 89.5%, B 100%) of the patients. In this cohort, 55.5% (W 43.4%, H 68.4%, B 50%) died at home with palliative care and 41% (W 46.3%, H 26.3%, B 50%) died in hospice facility. Three patients (all W, 2.4%) died in nursing home and two patients (one each W and H) died at hospital.

Conclusions: These data suggest that palliative care at the end of life was relatively well managed in all ethnic groups of patients with ALS. The finding that relatively more Hispanic patients opted for permanent ventilatory support and preferred to die at home needs further study on a larger number of patients.

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